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REVIEW

Plant-derived nanovesicles as an emerging platform for cancer therapy

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Abstract Plant-derived nanovesicles (PDNVs) derived from natural green products have emerged as an attractive nanoplatform in biomedical application. They are usually characterized by unique structural and biological functions, such as the bioactive lipids/proteins/nucleic acids as therapeutics and targeting groups, immune-modulation, and long-term circulation. With the rapid development of nanotechnology, materials, and synthetic chemistry, PDNVs can be engineered with multiple functions for efficient drug delivery and specific killing of diseased cells, which represent an innovative biomaterial with high biocompatibility for fighting against cancer. In this review, we provide an overview of the state-of-the-art studies concerning the development of PDNVs for cancer therapy. The original sources, methods for obtaining PDNVs, composition and structure are introduced systematically. With an emphasis on the featured application, the inherent anticancer properties of PDNVs as well as the strategies in constructing multifunctional PDNVs-based nanomaterials will be discussed in detail. Finally, some scientific issues and technical challenges of PDNVs as promising options in improving anticancer therapy will be discussed, which are expected to promote the further development of PDNVs in clinical translation.

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1. Introduction

Among all diseases, cancer is one of the deadliest diseases in the world. Current clinical treatment option mainly includes surgery, chemotherapy, radiotherapy (RT), immunotherapy, gene therapy (GT), high intensity focused ultrasound (HIFU) therapy, magnetic hyperthermia (MHT), as well as the newly emerged photodynamic therapy (PDT) and photothermal therapy (PTT)[1−9]. Amongst, surgery is essential for global cancer and more than 80% of cases will need surgery, but how to achieve safe, timely and efficient cancer surgery is challenging[10,11]. Other therapeutic modalities rely heavily on the utilization of therapeutic agents (*e.g.*, chemotherapeutic drugs, photosensitizer, radiosensitizer, magnetic hyperthermal substances) to cause cytotoxic effect on diseased tumor cells[12−15]. Unfortunately, the direct use of these conventional ("free") drugs is usually limited by some intricate problems, such as poor solubility, tissue damage during extravasation, poor biodistribution, adverse pharmacokinetics, non-selectivity for target tissues, and rapid breakdown[16,17]. Moreover, drug resistance is often reported[18,19]. All these limitations may lead to a remarkably reduced therapeutic efficacy or even the treatment failure. To this end, enormous research efforts have been devoted to searching for advanced techniques, strategies and materials for fighting against cancer over the past decades.

Fueled by the advances in biotechnology and materials science, nanotechnologies have been widely investigated for cancer treatment, aiming to enhance safety, accuracy, and efficacy by leveraging the unique properties of engineered nanomaterials[20,21]. These materials are usually designed in the form of nano- and/or microparticles to alter the biodistribution and pharmacokinetics of the therapeutic agents they are linked with, or to serve as drug reservoirs, or both[22,23]. By taking advantage of the enhanced permeability and retention (EPR) effect [that is a pathophysiological phenomenon of solid tumor vasculature caused by defects in vascular structure, gaps between vascular endothelial cells, abundant vascular mediators and impaired lymphatic recovery, leading to preferential filtration and permeation of nano-sized materials (10−100 nm) within tumors[24,25] as well as the multiple functions of nanomaterials (*e.g.*, active targeting, stimuli-responsiveness, high drug encapsulation), it is expected to overcome the physicochemical limitations of free drugs (*e.g.*, stability and solubility) and surmount the biological hurdles involved in drug delivery process (*e.g.*, blood circulation, immune clearance, off-target deposition and cell entry)[26−29]. Until now, a multitude of organic (*e.g.*, polymeric micelles, liposomes, dendrimers) and inorganic nanoparticles (*e.g.*, mesoporous silica nanoparticles, metal organic frameworks, gold nanoparticles) have been engineered for targeted cancer therapy, some of which are currently in preclinical studies or clinical trials, or even have been approved by the U.S. Food and Drug Administration (FDA)[30−35]. However, it still remains challenge to achieve successful tumor treatment owing to some critical limitations. For example, most of the synthetic nanomaterials usually need complex synthetic processes with the utilization of abundant regents and chemicals, which makes the acquisition of desired, highly purified products extremely difficult and may bring about unexpected side effects from the impurities. Moreover, synthetic nanomedicines lack the ability to interact autonomously with target tissues and/or diseased cells to achieve efficient therapeutic efficacy, which further increases the complicacy of design and construction of versatile nanomaterials with multiple functions to meet task-specific applications in the complex biological system. To this end, natural particulates originating from biological systems have evolved with key features (*e.g.*, self markers, antigenic components, physicochemical properties, and cell entry mechanisms) as newly emerging biomaterials for efficient tumor therapy.

Amongst, plant-derived nanovesicles (PDNVs) are phospholipid bilayer membrane-enclosed nanovesicles isolated from plants. In contrast to synthetic nanomaterials, PDNVs, as a kind of naturally occurring products and messengers for intercellular communication and cross kingdom regulation between plants, animals, and microorganisms, represent an excellent candidate for tumor growth inhibition in biomedical application[36,37]. They are constructed of lipid bilayers containing various bioactive components (*e.g.*, proteins, nucleic acids and other components) inside and membrane proteins on the surface. Their composition may vary depending on the derived plant. PDNVs are usually produced by plant cells (*e.g.*, grape, lemon, broccoli, strawberry, and ginger), especially those edible plants, rendering them as sustainable, cost-effective, and renewable naturally derived nanoplatforms[38−41]. Specifically, PDNVs are usually localized in the cytoplasm or spread into the extracellular space to function as intra- or inter-cell communicating media[36,42,43]. Depending on their original sources, PDNVs can possess different characters and biological functions, owing to their complex composition and bioactive contents inherited from the parent cells[44−46]. Growing evidence indicates that PDNVs carry numerous proteins, miRNAs, lipids, and metabolites (*e.g.*, amino acids, polysaccharides, vitamins, co-enzymes, and organic acids), some of which may elicit antifungal and antimicrobial activity, or exert anti-oxidant and anticancer effects, or regulate the genes between different organisms and species (Fig. 1)[47−50]. These biogenetically derived components and functions can be transferred by PDNVs in cell-to-cell communication[51−53]. In addition, the phospholipid bilayer membrane structure of PDNVs provides the opportunity for

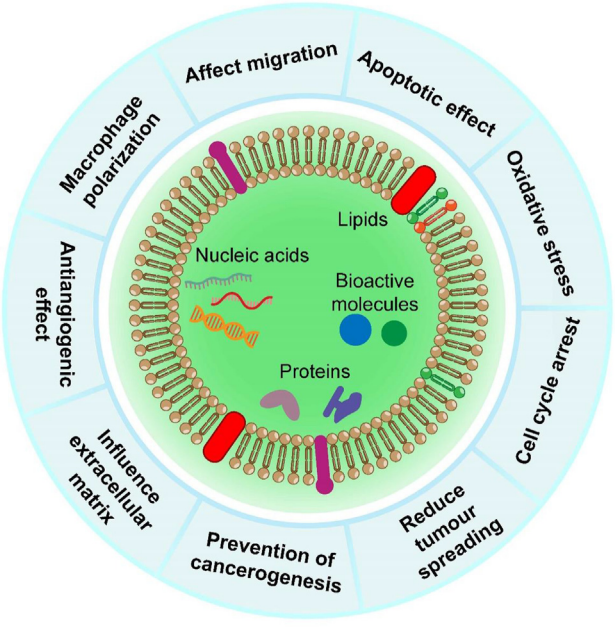

Figure 1 Bioactive components contained in PNDVs and their biological properties to take an effect on the occurrence, development and metastasis of tumors by acting on tumor cells or cells in the tumor microenvironment (TME), or even by modulating the TME.

carrying high amounts of extraneous cargos (*e.g.*, drugs, inhibitors, genes, proteins) and easy permeabilizing in cell compartments, rending them as excellent natural or bioengineered transportation vesicles for controlled release of drugs[54,55]. More importantly, unlike synthetic nanomaterials that usually needs complex separation and purification, PDNVs are a kind of green and biocompatible materials, which can be produced from natural plants, especially those edible plants (*e.g.*, lemon, ginger, corn). As such, these naturally occurring PDNVs would not generate a high toxic effect on biological systems, even if they may possess a certain content of exogenous molecules (*e.g.*, ginseng terpene and safrole, etc.)[56–58]. Since PDNVs serve as a bridge for communication between different kinds of cells, this makes it possible to have the ability to interact with human cells (especially tumor cells). Examples include the communication of PDNVs from ginseng and tea leaf with macrophages and PDNVs from lemon with tumor cells[59–61]. In addition, PDNVs naturally have a diameter of 50–500 nm without modification, making them suitable for *in vivo* biological applications. Also, their relatively simple source, often easily accessible plants, makes PDNVs suitable for bulk preparation. PDNVs also have high stability and can be maintained at 4 °C for over a month, meaning that they are suitable for clinical preservation[62].

Therefore, PDNVs offer a variety of advantages over synthetic nanomaterials, including low risk of toxicity, eco-friendly, sustainability, inherent inclusion of biological/chemical/physical functionality, biocompatibility, biodegradability, and easily obtainable sources. Owing to these conspicuous features, PDNVs have been developed as innovative nanomedicines for therapeutic application, especially in anticancer therapy.

In view of the important value of PDNVs as newly emerging and versatile nanostructures, this review is to gain insight on the application of PDNVs as therapeutic nanoplatforms for highly efficient cancer therapy. The characteristics, isolation methods, and structure of PDNVs used in cancer therapy will be included. Subsequently, the inherent anticancer properties of some kinds of PDNVs for achieving efficient antitumor effect will be presented in this review. In particular, emphasis will be focused on the use of engineered PDNVs with multiple functions as promising drug delivery systems, and representative nanostructures will be addressed in depth, which will open tremendous prospects for the design and construction of PDNVs-based nanomedicine in the future. Finally, the potential challenges and future perspectives in this field will be provided.

2. Plant-derived nanovesicles

2.1. *Methods for preparing PDNVs*

Current approaches to isolate PDNVs include size exclusion chromatography, ultrafiltration and differential ultracentrifugation, etc.[63–67]. Amongst, differential ultracentrifugation is the most popular technique for PDNVs' isolation and purification due to its recovery, specificity, low cost and suitability for large sample processing[68–71]. As the technique flow shown in Fig. 2, plant juice is firstly obtained by blending in a mixer or squeezing, which is preferably performed at 4 °C[72,73]. After filtering and removing large residues from the collected juice, cells and cell fragments will be eliminated by centrifugation at a low speed of 1000 $\times g$–5000 $\times g$. To further remove cells and cell debris completely, this step can be repeated several times by gradually increasing speeds and times. Then, the supernatant obtained from last step is further centrifuged at 10,000 $\times g$ to precipitate microvesicles (MVs) and organelles. The supernatant is finally centrifuged by using an ultracentrifuge at a speed of 100,000. 150,000 $\times g$. During this step, the PDNVs will be aggregated and ready to be suspended in PBS at the bottom of the centrifuge tube. To remove protein aggregates, nucleic acids and other contaminants, density gradients are centrifugated at a speed of 100,000 $\times g$–150,000 $\times g$ with gradient medium using 8%, 30%, 45% and 60% sucrose in PBS or Tris–HCl. PDNVs will then float in the sucrose band and can be extracted in their specific density region.

Supernatant from such an isolation approach contains not only extracellular vehicles (EVs), but also small amount of artificial nanoparticles/microparticles, which are generated by reassembly of fragmented cell membranes, as well as the native intracellular vesicles that are released during cells rupture[74,75]. At present, there is no available method to discriminate between authentic plant EVs and intracellular or artificially generated vesicles, which is why it is more accurate to call them PDNVs[75,76].

Moreover, ultrafiltration, which uses nano-porous membranes to filter samples through fluid pressure based on their molecular weight, was also tried as a method of isolating PDNV[77,78]. Ultrafiltration uses semi-permeable membranes with a defined molecular weight or pore size for isolation[79]. Low molecular weight particles and proteins can pass through the membranes, while high molecular weight components will be retained. Compared to ultracentrifugation, ultrafiltration is more efficient, simpler to operate, best retains the morphology of PDNVs, and it does not require complex equipment. However, it still faces problems, such as the highest protein contamination and a great loss of PDNVs attached to the membrane[80].

Also, size-exclusion chromatography (SEC) can isolate PDNVs based on particle size and molecular weight[81]. The stationary phase of the SEC consists of a porous polymer with a mobile phase that passes through the SEC column. Small particles, such as free proteins and nucleic acids, slow down by entering the pores of the polymer, while PDNVs, which are larger than the pores of the polymer, travel faster along the column and are therefore rapidly expelled in the early fractions[82]. Even through this method has good isolation effect and a wide range of eluates, it requires special equipment, a long running time, and is difficult to produce on a large scale[83,84]. Optimization of these isolation methods is crucial for obtaining PDNVs with specific size and morphology efficiently, conveniently, and environmentally.

In addition to the isolation techniques mentioned above, there are some unique purifying techniques developed to address some critical issues. For example, He et al.[29] found that the tetrapeptide (TET)-like gene expressed a tetraspanin-like protein called TET8 in Arabidopsis, and they subsequent used TET8 immunoaffinity capture to isolate PDNVs. During this process, beads coated with an antibody were used to recognize the particular nanovesicles-enriched surface proteins, so as to obtain a specific subclass of nanovesicles, which represent a powerful tool for PDNVs isolation.

What's more, PDNVs can also be isolated from plants by using the natural negative charge on the surface of PDNVs through the combination of electrophoresis and dialysis[85]. A dialysis bag containing plant juice is placed in the electrophoresis device. Under the electric field, contaminants, such as nucleic acids and proteins, pass out of the dialysis bag, and PDNVs are retained. All these methods provide abundant methodology option for

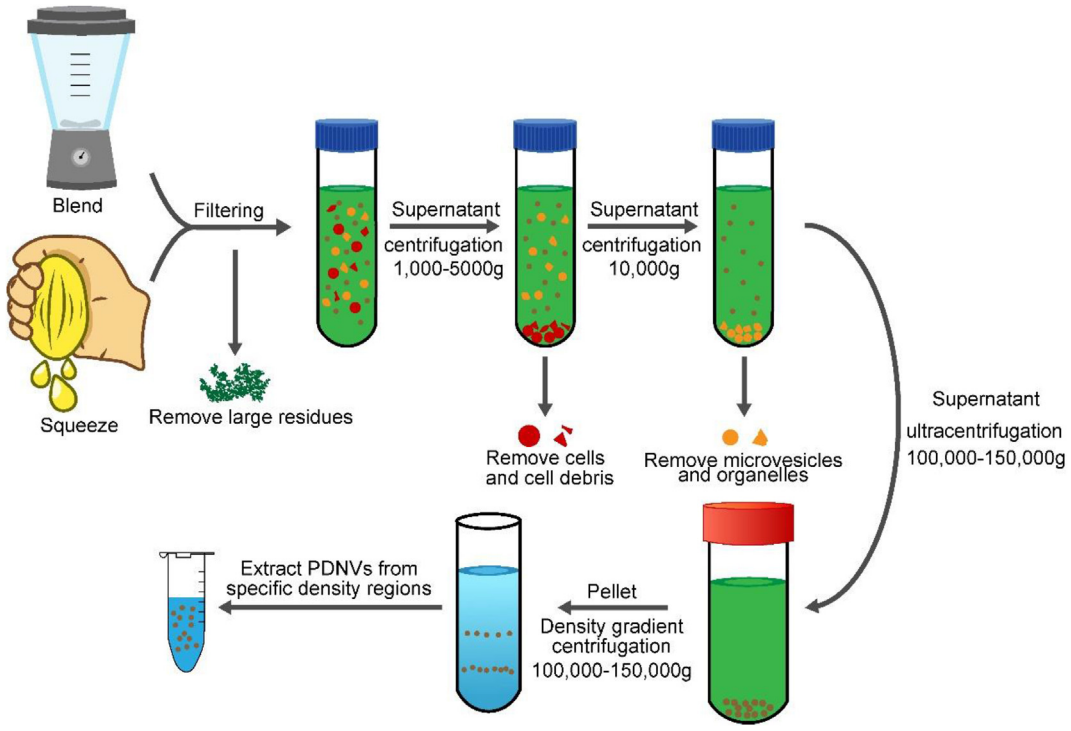

Figure 2 Technique flow for the isolation of plant-derived nanovesicles (PDNVs) by different times of differential centrifugation and ultracentrifugation.

reserchers to obtain desired PDNVs with complete structure and retained biological functions.

2.2. *Structure and composition of PDNVs*

The structural characteristics of PDNVs are much like those of EVs derived from animal or human cells. They are nano-sized particles (50−1000 nm) constructed by lipid bilayers membrane with various bioactive contents (*e.g.*, proteins, nucleic acids, and other components) within the interior and membrane proteins on the surface[86]. Depending on the plant species and isolation method, PDNVs can acquire a negative surface charge and a spherical, ellipsoidal, or cup-shaped morphology[87,88]. The naturally small size, regular morphology and negative surface charge of PDNVs endow them with good dispersion and long circulation times, making them suitable for biological applications. In general, the composition of PDNVs from different species of plants varies and mainly contains lipids, proteins, nucleic acids and others depending on their parent sources.

2.2.1. *Lipids of PDNVs*

Lipids are the key constituents of PDNVs. Some lipids, such as phosphatidic acids (PA), phosphatidylcholine (PC), phosphatidylethanolamines (PE), digalactosyldiacylglycerol (DGDG), monogalactosyldiacyglycerol (MGDG), phosphatidylinositol (PI), monogalactosyl monoacylglycerol (MGMG), diacylglycerol (DAG), triacylglycerol (TAG), have been identified in different kinds of PDNVs[89−92]. It has been reported that lipids, such as PA, are considered to be the most important cellular sensor of essential nutrients for regulating cell proliferation and has been identified as the necessary component to build the stable membrane-based vesicles and maintain the integrity of PDNVs[93,94]. The lipid content and lipidic structural assembling of PDNVs are connected to both their function and ability to be quickly taken up by cells. A study showed that PA-rich ginger-derived nanovesicles sent signals to *Lactobacillus rhamnosus* and gave rise to preferential absorption, thereby altering the physiology of the host as well as the composition and location of the bacteria[95]. In addition, Deng et al.[96] demonstrated that lipids of broccoli-derived nanovesicles could induce increased populations of tolerogenic dendritic cells (DCs), and activation of adenosine monophosphate-activated protein kinase (AMPK) in DCs that exhibited an anti-inflammatory effect in colitis mice.

2.2.2. *Proteins of PDNVs*

Proteins in PDNVs play an indispensable role in plant immunity[97]. A large amount of proteins, such as 3129 and 1018 proteins, are found in PDNVs from ginseng and citrus clementina fruit, respectively[59,98]. Nanovesicles isolated from strawberry contain more than 200 different proteins, among which heat shock proteins (HSPs) are often detected in PDNVs[99]. In addition, different types of HSPs are found in PDNVs like citrus fruit, grapefruit and tomato[100−102]. What's more, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), an essential glycolytic enzyme, is also found as one of the most abundant proteins in PDNVs from grape, citrus and grapefruit[100,101,103]. In general, the current studies on the proteins of PDNVs indicate that they are inferior to mammalian EVs in terms of quantity and complexity[104,105].

2.2.3. *Nucleic acids of PDNVs*

PDNVs contain a large variety of different types of nucleic acids, such as small RNAs (sRNAs) that include microRNAs (miRNAs) and small interfering RNAs (siRNAs)[106−109]. These sRNAs play a key function in mediating intercellular communication as well as

treating inflammatory diseases and cancers[110−114]. Therefore, the analysis of nucleic acids in PDNVs is extremely important for figuring out the functional mechanism in human diseases and their possible therapeutic applications. In a study of nanovesicles derived from 11 vegetables and fruits, analyses indicated that a total of 418 miRNAs were identified, which were associated with cancer-related pathways and inflammatory responses[115]. Moreover, these miRNAs had the potential to regulate human mRNAs. In another study, nanovesicles from rice aleurone were demonstrated to be enriched with Hvu-MIR168-3p, which showed an ability to reprogram energy metabolism in human cells, upregulate protein and glucose transporter protein I RNA expression and ultimately decrease the blood glucose level[116].

2.2.4. *Other components of PDNVs*

Different PDNVs may contain different bioactive substances depending on their distinct origins. In many studies, the beneficial properties of PDNVs can be attributed to these bioactive molecules[117]. For example, strawberry-derived nanovesicles contain vitamin C, a natural free radical scavenger that protects not only plant cells, but also human mesenchymal stromal cells from oxidative stress[41]. An earlier study by Chen et al.[118] found that tea flower-derived PDNVs might contain a large number of polyphenols and flavonoids. They also revealed that tea leaf-derived PDNVs from three different leaf size species contained various types of polyphenols and flavones[119]. These bioactive substances have been reported to have multiple biological activities and may disrupt numerous signaling pathways involved in apoptosis, migration, immune response and dysfunction associated with the hallmarks of cancer[120,121]. The understanding of these biocomponents involving in the regulation of cancer cell biological behaviors might provide interesting insights on the design of novel anticancer therapeutics.

3. Applications of PDNVs for cancer therapy

Due to their physicochemical characteristics (*i.e.*, size, surface charge), PDNVs have been shown in *in vitro* enzymatic digestion and *in vivo* evaluation studies to be stable in gastric and/or intestinal simulated fluids, suggesting their potential as nutrient carriers and drug delivery systems when vegetables and fruits are used for food or oral administration[122,123]. In the regard of cancer therapy, these biocompatible and biostable PDNVs can be used either as therapeutics to transfer their own biological cargos (*e.g.*, nucleic acids, cell-surface proteins, bioactive lipids), or as active delivery vehicles to transport other therapeutic drugs to kill tumor cells specifically with less side effects[124,125].

3.1. *Inherent anticancer properties of PDNVs*

As mentioned above, PDNVs contain many different bioactive molecules, which have been demonstrated to reduce the risk of carcinogenesis and hold an antitumor effect. These substances are inextricably linked to the plants that they are derived from. Different plants contain a variety of different phytochemicals, many of which are their derived analogs, such as curcumin, gingerol, gallic acid, tannins, and others, and have been identified to have the potential of cancer treatment[126−130]. Therefore, these substances can be used as anticancer therapeutics by targeting a variety of cancer cell signaling pathways, inhibiting cancer cell activating proteins and enzymes, promoting apoptosis, inducing cell cycle arrest, and regulating antioxidant status[131−136]. It should be noted that different types of PDNVs have their own advantages and disadvantages owing to their distinct properties that play different roles in biological systems. For example, tea leaf-derived nanovesicles are rich in bioactive components such as polyphenols, which can exert powerful anti-inflammatory and antitumor activities, but have not been found to alter the immunosuppressive TME[60]. In contrast, ginseng-derived nanovesicles are rich in ginseng polysaccharides, which can improve the immunosuppressive TME by promoting macrophage M1 polarization, but do not exert anti-inflammatory effects[59]. In addition, lemon-derived nanovesicles were not found to have any effect on the immunosuppression of TME, but they could consume tumor cells' energy by enhancing cell uptake, thereby improving tumor multidrug resistance[61]. The typical features of different types of PDNVs are listed in Table 1[26,59,85,102,103,119,122,137−144].

Recently, Chen et al.[118] reported that tea flower-derived nanovesicles exhibited a strong cytotoxic effect on cancer cells (Fig. 3A). These nanovesicles contained high amounts of bioactive components, such as polyphenols and flavonoids. These components have been shown to increase the oxidative stress in breast cancer cells, which, in turn, triggered cell cycle arrest and severe mitochondrial damage, and ultimately exerted multiple anticancer functions (*e.g.*, anti-proliferation, anti-mobility, and pro-apoptotic in cancer cells). *In vivo* experiments revealed that these tea flower-derived nanovesicles either administered orally or intravenous injected could considerably inhibit the development and metastasis of breast tumors. Furthermore, PDNVs isolated from three different sizes of tea leaves could target macrophages through galactoses on their surface (Fig. 3B)[119]. This significantly reduce the inflammatory response, repair the disordered colonic barriers and maintain the homeostasis of gut microbiota in the bowel, thereby preventing or reducing inflammatory bowel disease (IBD) and colitis-associated colon cancer (CAC) in mice. Anti-inflammatory and cancer-preventive potential of PDNVs isolated from other plants have also been reported[145−147]. Some other innate active molecules, such as 6-gingerol, 6-shogaol, and naringin, also have the anticancer activity. For example, ginger-derived nanovesicles (GDNVs) containing high concentrations of active ginger constituents have been demonstrated to speed up intestinal repair and prevent the initiation of IBD and CAC[122]. Oral administration of GDNVs increased the production of anti-inflammatory cytokines, inhibited the level of pro-inflammatory cytokines, and promoted the proliferation and survival of intestinal epithelial cells, which ultimately promoted the mucosal healing. In addition to affecting the apoptosis-associated signaling pathways or damaging the critical intracellular organelles to induce cell death, the direct cell killing effect of PDNVs have also been demonstrated in previous studies. As an example, Raimondo et al.[137] demonstrated that lemon-derived nanovesicles (LDNVs) could inhibit the growth of chronic granulocytic leukemia through TRAIL-mediated apoptosis and the procession of tumor-associated angiogenesis. Furthermore, Yang et al.[85] showed that LDNVs isolated using electrophoresis and dialysis techniques could exert anticancer effects both *in vitro* and *in vivo* (Fig. 4A). This finding revealed that LDNVs promoted intracellular reactive oxygen species (ROS) generation and upregulated the expression of GADD45a in gastric cancer cells, which led to the cell cycle S-phase arrest and apoptosis (Fig. 4B and C).

Stanly et al.[138] reported that nanovesicles isolated from four kinds of *Citrus* species, *Citrus limon*, *Citrus sinensis*, *Citrus aurantium* and *Citrus paradisi*, could impair the proliferation of

Table 1 Representative PDNVs used in cancer therapy.

Origin	Size	Target	Feature	Administration	Ref.
Tea flower	131 nm	MCF-7 and 4T1 cells	Inhibit breast cancer growth and metastasis	i.v. injection and Oral	26
Tea leaf	134.0−145.6 nm	RAW 264.7 cells	Prevent or alleviate IBD and CAC	Oral	119
Ginger	219.6−292.5 nm	RAW 264.7 and Colon-26 cells	Faster intestinal repair and prevent the initiation of IBD and CAC	Oral	122
Lemon	50−70 nm	LAMA84, SW480 and A549 cells	Induce TRAIL-mediated cell death and inhibit tumor-associated angiogenesis	IT and IP injection	137
	~100 nm	AGS, BGC-823, and SGC-7901 cells	Increase ROS generation, induce gastric cancer cell cycle S-phase arrest and apoptosis	i.v. injection	85
	~136 nm	HCT-15, SW480, and HCT116 cells	Inhibits p53-inactivated colorectal cancer cells *via* the macropinocytosis pathway		103
Citrus species		A375 cells	Induce G2/M cell cycle arrests, promote apoptosis, and suppress the expression of various critical cancer hallmarks		138
Dendropanax morbifera, *Pinus densiflora*, *Thuja occidentalis,* and *Chamaecyparis obtusa*	83, 56, 114, 185 nm	A431, MCF7, and B16BL6 cells	PDNVs from DM and PD improve the cytotoxic effects		139
Dendropanax morbifera	100−200 nm	B16BL6 cells	Exert a concentration-dependent suppressive effect on CAFs and alter the expression level of genes in CAFs		140
Moringa oleifera	240−500 nm	Hela cells	Decrease the expression of proteins associated with apoptosis		141
Garlic	50−150 nm	A498 and A549 cells	Inhibit tumor-associated angiogenesis and induce caspase mediated apoptosis in tumor cells		142
Ginseng	344.8 nm	M0 macrophage, B16F10 and 4T1 cells	Trigger macrophage polarization in a TLR4/MyD88-dependent manner	IP injection	59
Petasites japonicus	122.6 nm	BMDCs	Strongly induce the maturation of DCs		143
Corn	80 nm	RAW264.7, colon26, and colon26/fluc cells	Inhibit the proliferation of colon26 cells and increase TNF-α production	IT injection	144
Cannabis	163.9 ± 27.16 nm 133.2 ± 5.75 nm	HepG2 and Huh-7 cells	Activates mitochondrial-dependent apoptosis signaling pathways		102

lung, skin and breast cancer cells without significantly affecting the growth of healthy cells. Further investigation showed that the possible mechanisms were related to the induction of G2/M cell cycle arrests, the promotion of apoptosis, and the suppression of the expression of several crucial cancer markers. Another group studied the cytotoxic effects of PDNVs extracted from *Dendropanax morbifera* (DM), *Thuja occidentalis* (TO), *Pinus densiflora* (PD), *and Chamaecyparis obtuse* (CO) (Fig. 5A)[139]. They found that whereas PDNVs from TO and CO had no obvious influence on any specific kind of cancer cell, those from DM and PD had a considerable cytotoxic effect on cancer cells. Additionally, they discovered a synergistic interaction between PDNVs from DM and PD to improve the cytotoxic effects through the growth inhibition and apoptosis induction. More recently, they further investigated the inhibitory effects of DM-derived nanovesicles against cancer-associated fibroblasts (CAFs) in a 3D microfluidic model and found that the nanovesicles could exert a concentration-dependent suppressive effect on CAFs and alter the level of gene expression in CAFs, particularly growth factor (*e.g.*, integrins) and extracellular matrix (*e.g.*, collagens)-related genes such as integrins and collagens (Fig. 5B)[140]. Potestà et al.[141] purified MVs from the aqueous extract of *Moringa oleifera* seeds

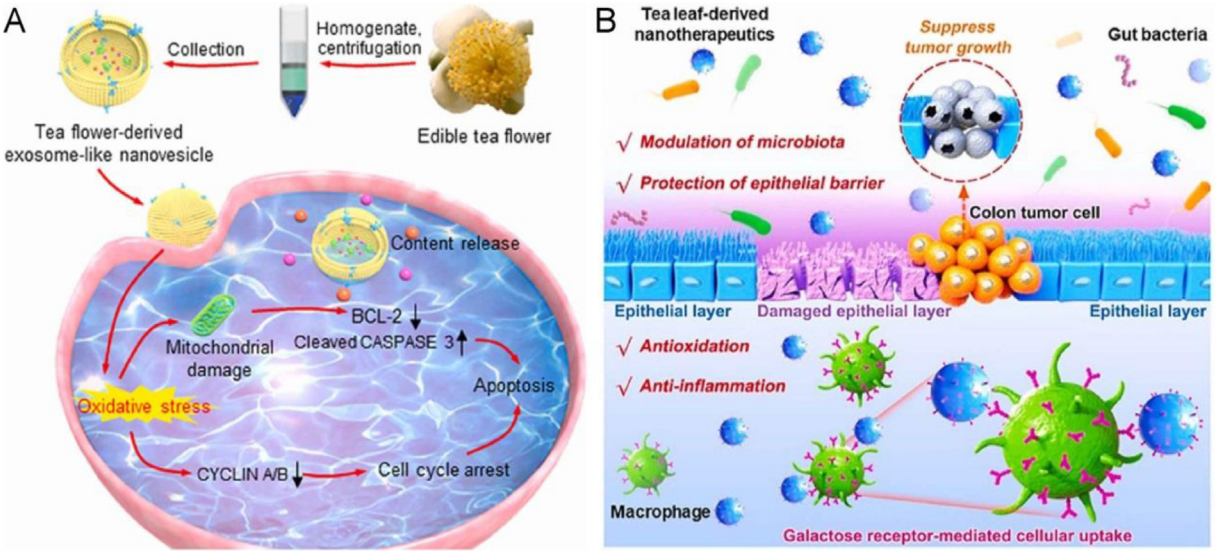

Figure 3 (A) Schematic illustration of the isolation and therapeutic functions of tea flower-derived nanovesicles *via* inducing oxidative stress, mitochondrial damage, cell cycle arrest and apoptosis. Adapted with permission from Ref. 46. Copyright © 2022 Elsevier. (B) Illustration of the preventive and therapeutic effects of tea leaf-derived nanovesicles on IBD and CAC through the ability to target macrophages *via* the galactose on the surface, the modulation of microbiota, protection of epithelial barrier, antioxidation, and anti-inflammation. Adapted with permission from Ref. 47. Copyright © 2021 Elsevier.

(MOES MVs) and found that MOES MVs could significantly increase the apoptosis of cancer cells, which was associated with a decreased BCL2 protein expression and reduced induction of mitochondrial membrane potential. Such effects were comparable between cancer cell lines treated with MOES MVs and those cell lines transfected with the pool of sRNAs extracted from MOES. More recently, garlic-derived nanovesicles were reported to display a severe cytotoxic effect on human kidney carcinoma and lung carcinoma cells[142]. Specifically, this kind of PDNVs could reduce the expression of soluble vascular endothelial growth factor (VEGF) protein, to inhibit the tumor-associated angiogenesis and induce caspase-mediated apoptosis in tumor cells.

In addition to their direct anticancer effects, PDNVs can also inhibit the tumor growth *via* evoking antitumor immunotherapy response in various ways[148,149]. As an example, Cao et al.[59] found that ginseng-derived nanovesicles could trigger macrophage polarization in a TLR4/MyD88-dependent manner. Thereafter, continued M1 polarization of macrophage enhanced the induction

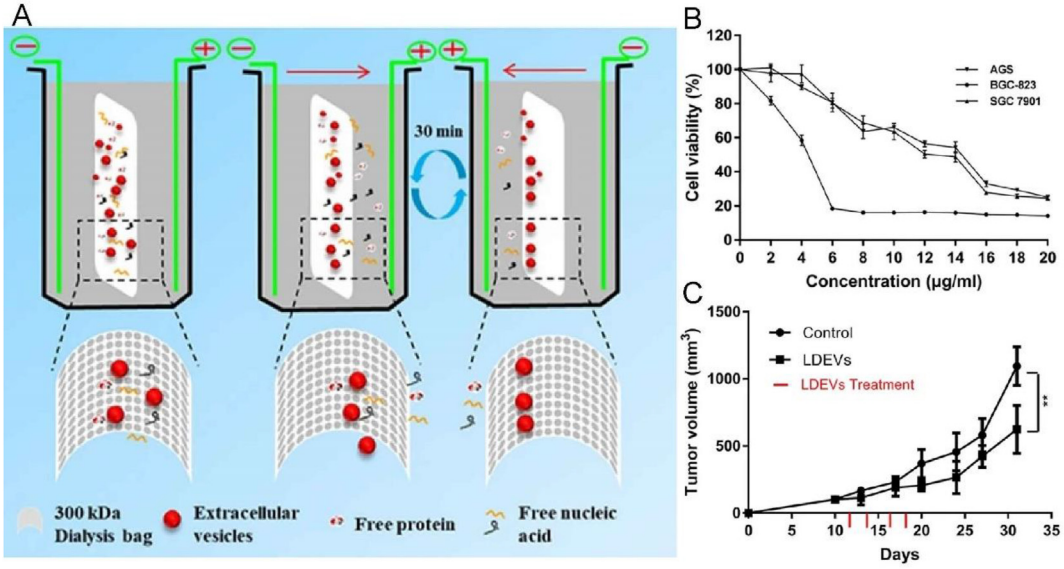

Figure 4 (A) Schematic of EVs isolation using ELD. (B) Cell viability of AGS, BGC-823 and SGC-7901 cells treated with different concentrations of LDEVs. (C) Tumor growth curve of the control and LDEVs treatment groups. Adapted with permission from Ref. 55. Copyright 2020 BioMed Central.

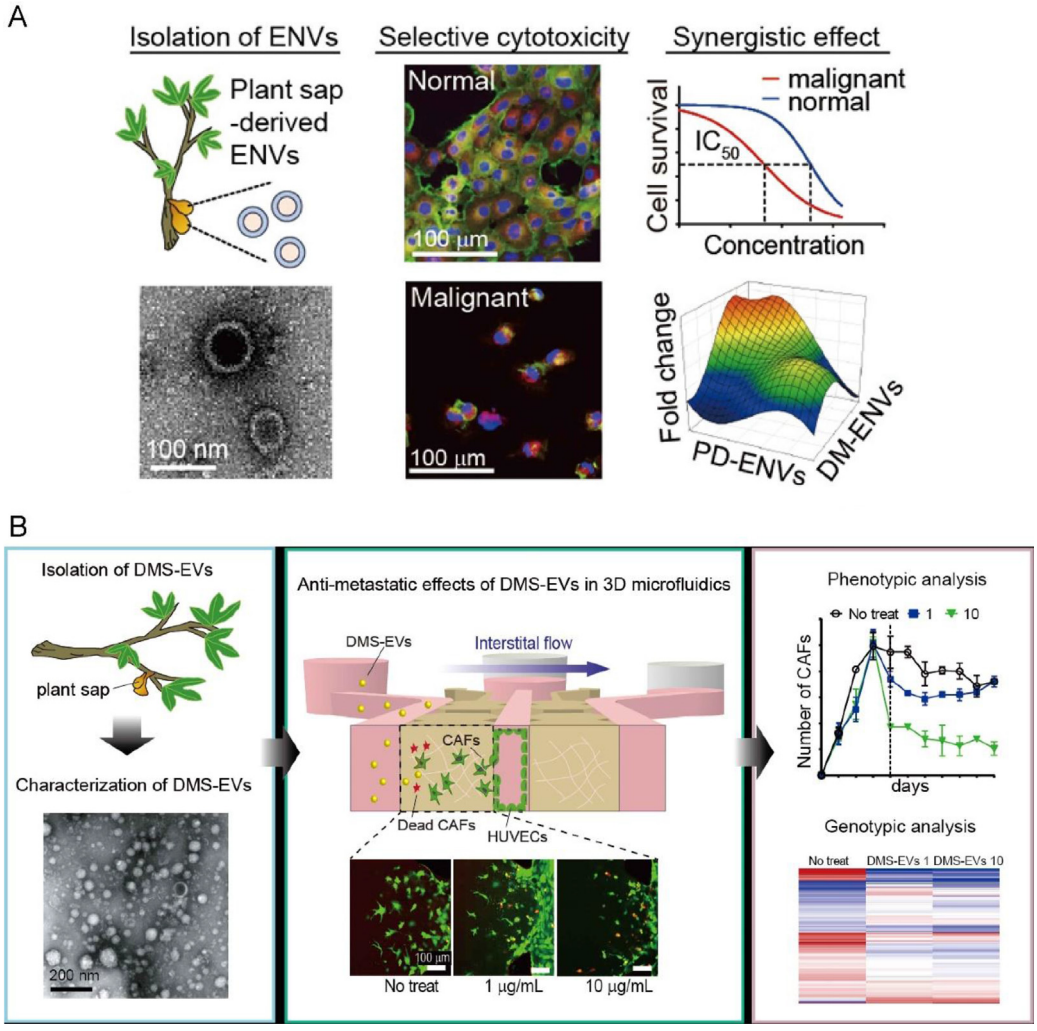

Figure 5 (A) Schematic of the isolation, selective cytotoxicity and synergistic effect of plant sap-derived extracellular vesicles (PD-ENVs). Adapted with permission from Ref. 57. Copyright 2020 Multidisciplinary Digital Publishing Institute. (B) Schematic of the isolation, characterization and anti-metastatic effects of DM-derived nanovesicles (DMS-EVs) in a 3D microfluidic cancer metastasis model. Adapted with permission from Ref. 58. Copyright 2020 Multidisciplinary Digital Publishing Institute.

of cancer cell apoptosis in TME, which in turn led to a significantly reduced tumor growth in mice inoculated with melanoma cells. In the study reported by Jeong et al.[143], *Petasites japonicus*-derived nanovesicles (PJDNVs) were demonstrated to dose-dependently increase the expression of surface molecules (*e.g.*, such as CD86, CD80, MHC-I, and MHC-II) on DCs in a large extent and upregulate the secretion of Th1 cytokines [*e.g.*, tumor necrosis factor (TNF)-α and IL-12]. What's more, they also found that PJDNVs could enhance the maturation and antigen-presenting ability of DCs and simultaneously decrease the antigen-uptake ability of these cells. As a result, PJDNVs-treated DCs significantly induced the proliferation and differentiation of naive T cells into Th1-type T cells and cytotoxic $CD8^+$-type T cells, as well as the massive secretion of Th1 cytokines [*e.g.*, interferon (IFN)-γ and interleukin (IL)-2], which were critically crucial factors for the induction of anticancer immunity. A recent study showed that corn-derived nanovesicles remarkably reduced tumor growth in mice by suppressing the proliferation of colon cancer cells and increased the production of TNF-α by activating macrophages and other immune cells infiltration into the tumor, both of which have a remarkable synergistic or additive effect on the anticancer therapy[144]. Collectively, these examples suggest that certain kinds of PDNVs have great potential for eliciting immune response to mediate tumor development and inhibit the growth of tumor, rendering them as a robust natural nanoplatform for the treatment of malignancies.

3.2. *PDNVs-based drug delivery systems*

3.2.1. *Loading therapeutic agents directly into PDNVs*

In addition to the direct application of PDNVs as therapeutic agents, PDNVs also provide a superb platform for creating drug/gene/protein delivery therapeutic carriers with ideal performance, so called drug delivery systems (DDSs), owing to their unique structures and naturally harmless traits (Table 2[61,150-160]). The main advantages of PDNVs-based DDSs lie in their long-term

Table 2 Engineered PDNVs *via* different methods for enhanced tumor therapy.

Origin	Size	Loaded drug	Modification	Target	Feature	Administration	Ref.
Bitter melon	100−200 nm (before loading)	5-FU		CAL 27 and WSU-HN6 cells	Improve the therapeutic efficacy of 5-FU and reduce drug resistance	Peritumoral injection	150
Cabbage and red cabbage	98.8−148.2 nm (ultrafiltration and size-exclusion chromatography, before loading) 100 nm (size-exclusion chromatography, before loading)	MiRNAs or DOX		SW480 cells	MiRNAs could be encapsulated in Cabex and delivered to colon cancer cells, DOX could be encapsulated in Rabex and Cabex and effectively inhibited colon cancer cell proliferation		151
Lemon	151.63 ± 5.20 (before loading) 202.20 ± 6.21 nm (after loading)	DOX	Functional heparin-cRGD	SKOV3 and A2780 cells	Inhibit the efflux function of P-gp, thus efficiently overcoming the multidrug resistance of DOX-resistant ovarian cancer	i.v. injection	61
Grapefruit	156 ± 33 nm (NaCl, after loading) 125 ± 22 nm (H_2O, after loading) 188 ± 28 nm (PBS, after loading)	MiR-18a		Kupffer and CT26 cells	Polarize Kupffer cells to M1 cells and induce the generation of IL-12	i.v. injection	152
	~30 nm (before loading) 43 ± 15 nm (after loading)	BSA or HSP70		HCT116, DLD1 cells, and PBMCs	Ameliorate the uptake of exogenous proteins	i.v. injection	153
	180.6 nm (before loading)	Chemotherapeutic agents, siRNA, DNA expression vectors or proteins	Liposomes, Folic acid	GL26, A549, SW620, CT26, and 4T1 cells	Enhance targeting efficacy of chemotherpy on tumors in a physiological milieu	i.v. injection	154
		DOX or curcumin	Coated with inflammatory related receptor enriched membranes of activated leukocytes	EL4, 4T1, 4TO7, and CT26 cells	Inhibit breast and colon tumor cell growth and attenuate DSS-induced colitis	i.v. injection	155
	72.4−102.4 nm (before loading) 87.2 ± 11.3 nm (after loading)	MiR17	Hybrid with polyethylenimine, coated with FA	CL-26 cells	Taken up by brain GL-26 tumor cells and inhibite GL26 tumor growth	Intranasal administration	156
	135 ± 5 nm (before loading) 192 ± 7 nm (after loading)	DOX	Surface modification using doxorubicin-loaded heparin-based nanoparticles	DOX-resistant MCF-7, LN929, U251, and U87	Bypasses BBB/BBTB and greatly promotes cell internalization and anti-proliferative ability	i.v. injection	157

(*continued on next page*)

Table 2 (*continued*)

Origin	Size	Loaded drug	Modification	Target	Feature	Administration	Ref.
Ginger	~188.5 nm (before loading)	DOX	Folic acid	Colon-26 and HT-29 cells	Inhibite the tumor growth in a Colon-26 xenograft model	i.v. injection	158
	123.5 nm (before loading)	Survivin siRNA	FA conjugated with arrowtail RNA	KB cells	Silence the survivin gene expression and suppress tumor growth	i.v. injection	159
Asparagus cochinchinensis	92, 119, 179 nm (before modification) 108 ± 17 nm (after modification)		PEGylation	Hep G2, SMMC-7721, and Hep 3B cells	Induce cellular apoptosis by upregulating apoptosis-related factors in hepatocellular carcinoma cells	i.v. injection	160

storage and high stability under physiological conditions, allowing them to circulate in body fluids for a long period of time[48,161]. Moreover, their high biocompatibility, ease of mass production, and highly efficient cellular uptake by cancer cells also make PDNVs as suitable candidates for drug delivery[76].

Until now, various exogenous therapeutic molecules, such as proteins and chemotherapeutic drugs, have been attached to or encapsulated in PDNVs[101,157]. The approaches of incorporating therapeutic molecules into PDNVs can be divided into two main categories: passive and active loading. Passive loading is relatively simple by incubating therapeutic agents with PDNVs for a period of time, and the drug agents will naturally diffuse into the inner cavity of PDNVs along the concentration gradient[162,163]. However, the loading capacity of passive method is much lower than that of active loading[164]. Therefore, diverse active loading methods are developed, which include the processes of sonication, incubation with membrane permeabilizers, freeze/thaw cycles, electroporation and extrusion[165–169]. In this way, the drug loading efficiency can be remarkably improved, and large molecules, such as proteins and RNAs, can also be loaded into PDNVs[170,171]. One of the main purposes of PDNVs carriers is to prevent the potential adverse effects of therapeutic drugs on normal healthy cells or tissues. In addition, PDNVs-based DDSs exhibit a low level of immunogenicity. The size and lipid membrane of PDNVs make them ideal for fusion with target cells, which can help to avoid the unspecific degradation of cargo before reaching its target and thus deliver it better to its destination[172]. As an example, Yang et al.[150]found that bitter melon-derived nanovesicles (BMNVs) possessed the ability to stimulate ROS generation for inducing S phase cell cycle arrest and apoptosis on oral squamous cell carcinoma cells (Fig. 6A and B). Based on their previous study that the increased intracellular ROS could activate NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3), which was associated with the resistance to 5-fluorouracil (5-FU)[173], they further demonstrated the downregulation of NLRP3 expression by the use of BMNVs. Thereafter, they used BMNVs as drug delivery vesicles to load 5-FU by sonication and found that 5-FU-loaded BMNVs decreased the expression of NLRP3 and IL-1β significantly, which was mediated by the increased ROS production. As a result, a much-enhanced cytotoxicity of 5-FU was shown in both *in vivo* and *in vitro* experiments compared with that of free 5-FU, demonstrating a much-reduced effect of drug resistance of 5-FU on OSCCs for achieving an improved therapeutic efficacy (Fig. 6C and D). Recently, You et al.[151] investigated the biological activities of nanovesicles derived from cabbage (Cabex) and red cabbage (Rabex) in human cells as novel therapeutic drug delivery vehicles (Fig. 7A). Their results showed that Cabex could load fluorescent dye-labeled anti-sense DNA oligonucleotides and deliver them to human cells (Fig. 7B). Based on these results, miR-184 was further encapsulated in Cabex with a high loading efficiency and delivered successfully to colon cancer cells (Fig. 7C). Thereafter, they additionally encapsulated a chemotherapeutic drug [*i.e.*, doxorubicin (DOX)] into Rabex and Cabex by incubation and successfully delivered the loaded cargo to cancer cells, resulting in effectively inhibited colon cancer cell proliferation.

In a work reported by Teng et al.[152], grapefruit-derived nanovectors (GNVs) were demonstrated to have the ability of loading miR-18a, and the delivered miR-18a was able to polarize Kupffer cells to M1 cells and induce the generation of IL-12 from M1 IFNγ^+ cells, which could subsequently cause immune cells [*i.e.*, NK (natural killer) and NK T cells] to activate and inhibit the

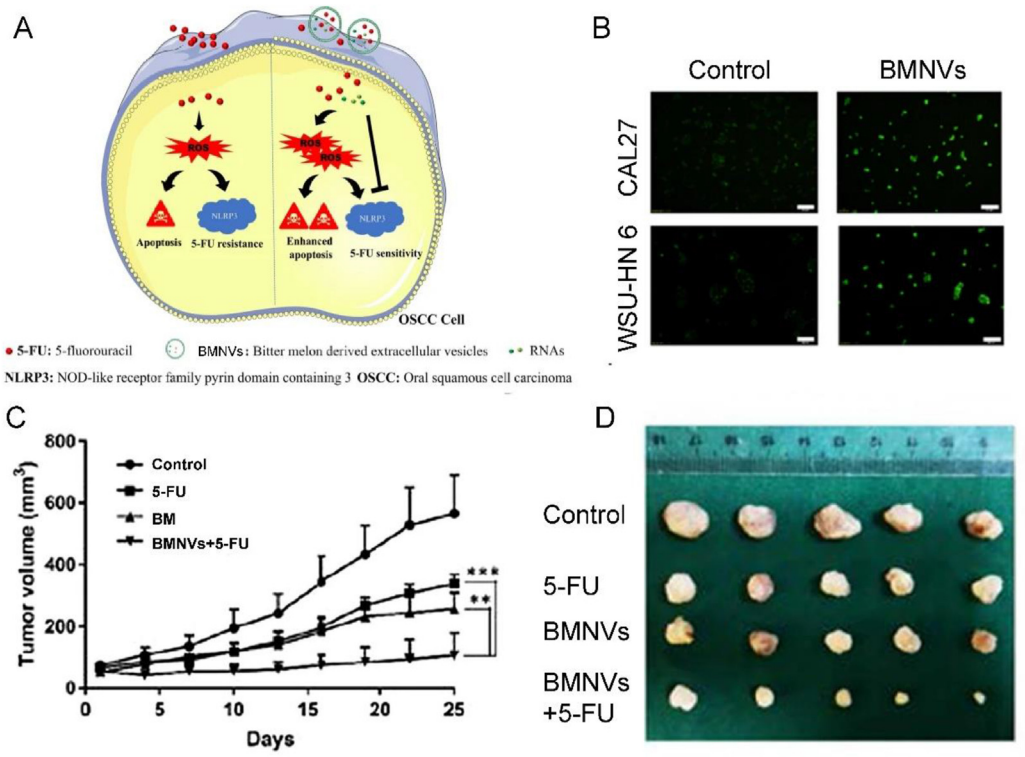

Figure 6 (A) Schematic diagram of the therapeutic mechanism of 5-Fu, bitter melon-derived nanovesicles (Group: BMEVs), and 5-FU-loaded bitter melon-derived nanovesicles (Group: BMNVs + 5-Fu). (B) Fluorescence images of the intracellular ROS levels after BMNVs treatment, scale bar = 200 μm. (C) Tumor growth curve of the control, 5-FU, BMNVs and BMNVs + 5-FU groups. (D) Representative images of tumors. Adapted with permission from Ref. 71. Copyright 2021 BioMed Central.

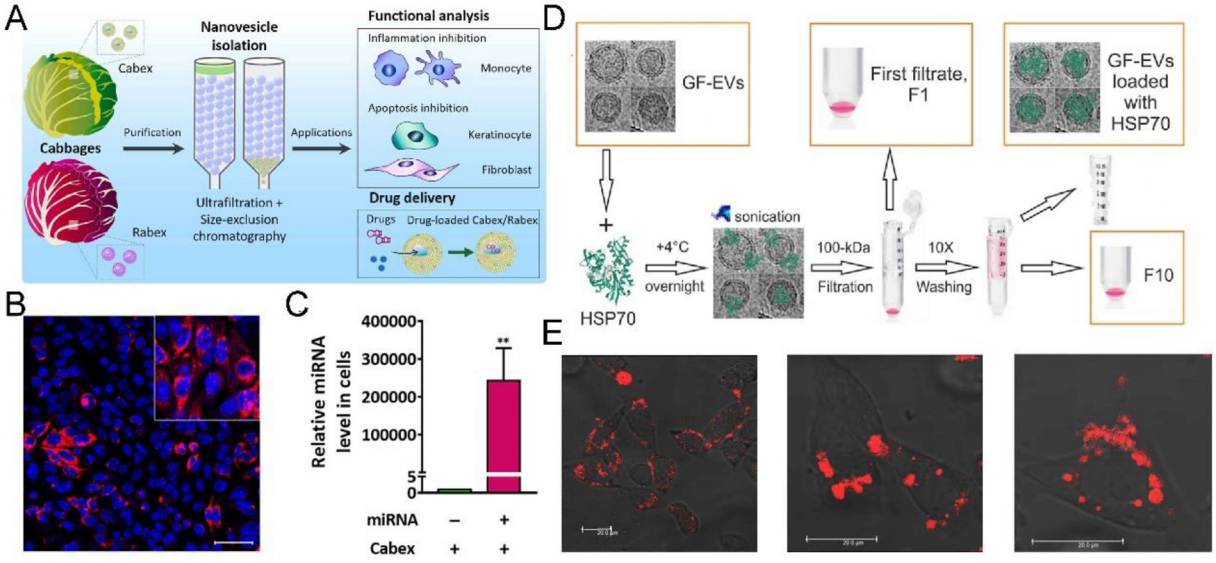

Figure 7 (A) Schematic diagram of nanovesicles isolation from cabbage and the functional analysis and drug delivery of Cabex and Rabex. (B) Fluorescence microscopy image of Cabex-delivered DNA oligonucleotides (red). Hoechst 33342 was used for nuclei staining (Blue). Scale bar indicates 50 μm. (C) Cell viability of SW480 cells after treatment with Cabex loaded with DOX. Adapted with permission. Adapted with permission from Ref. 73. Copyright © 2021 Elsevier. (D) Schematic of the preparation of HSP 70-loaded grapefruit-derived nanovesicles (GF-EVs). (E) Fluorescence micrograph of DLD1 cells co-cultured with GF-EVs loaded with HSP70-AF647. Adapted with permission from Ref. 75. Copyright 2021 Nature Publishing Group.

growth of metastatic colon cancer cells in the liver. This kind of PDNVs were demonstrated as an effective therapeutic vehicle to deliver therapeutic RNA with high capacity for the treatment of liver metastasis of malignancy.

In addition to transferring innate protein to recipient cells and inducing diverse cellular functions, PDNVs can also deliver exogenous proteins. Garaeva et al.[153] reported that grapefruit-derived nanovesicles with an average size of ~ 41 nm were highly efficient carriers to deliver the exogenous bovine serum albumin and HSP 70 into human cells (*i.e.*, human colon cancer cells and peripheral blood mononuclear cells) *in vitro* (Fig. 7D). As a result, the cellular uptake of exogenous proteins could be significantly ameliorated and the function activity of these proteins could be well maintained, demonstrating the significant importance of using grapefruit-derived nanovesicles (Fig. 7E).

3.2.2. *Multifunctional engineering of PDNVs*

Compared to the direct use of bare PDNVs as vesicles for drug delivery, re-engineering PDNVs-based carriers will endow the nanoplatform with various functions (*e.g.*, "stealth" property, targeting ability, deep tumor penetration, imaging and detection) to achieve highly efficient drug delivery and desired therapeutic outcome[174–176]. These functions can help the delivery system to cross the biological barriers and surmount the critical challenges encountered in drug delivery process, such as the fast elimination by the reticuloendothelial system (RES), macrophage internalization, the non-specific uptake by normal cells, adsorption of serum proteins[177–179]. For example, to achieve a prolonged blood half-life time of nanoparticles in body system, their surfaces are usually functionalized with a stealth coating layer using biocompatible polymer, such as zwitterionic polymers, poly (ethylene glycol) (PEG), polyanions [*e.g.*, poly(aspartic acid)][180,181]. Amongst, PEGlation has been regarded as an ideal standard polymer for modifying particles owing to its hydrophilic nature, nontoxic property, and steric exclusion effect to repel protein adsorption, so as to dramatically increase the circulation time, which in turn helps the nanoparticles to accumulate at tumor site[182–184]. At the same time, the stealth outer layer can also enhance the colloid stability and water solubility of nanoparticles. In order to enable the PDNVs-based DDSs to recognize diseased tumor cells, targeting ligands (*e.g.*, peptides, folic acid, antibodies, hyaluronic acid) are usually modified on the surface of nanovesicles for selectively recognizing and combining with particular markers on the surface of cancer cells[185–193]. In this way, the drug-loaded carriers can reduce the uptake by healthy tissues/cells and actively target specific tumor cells, which will promote the aggregation of nanoparticles at tumor sites and enhance the intracellular drug concentration for efficient tumor cell killing. Moreover, a combinatorial therapeutic effect can be achieved by loading more than one therapeutic agent into a single nanoplatform, and the function of therapy and diagnosis can also be realized at the same time *via* the integration of imaging agents with drug molecules[194]. With these points in mind, various re-engineered PDNVs-based DDSs have been constructed as function-enhanced therapeutic platforms for high performance cancer therapy.

As a kind of biomembrane-based nanostructure, there are several ways to integrate multiple functions into a single PDNVs platform (Fig. 8). Physical adsorption, chemical grafting, and membrane insertion has been widely used for surface modification[195–197]. As a typical example, the copper-free "click chemistry" has been regarded as a powerful tool to perform

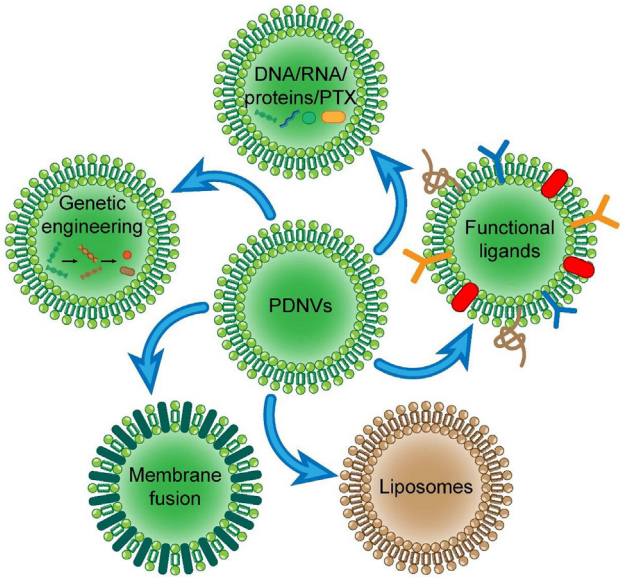

Figure 8 Several ways of engineering PDNVs.

nanovesicles modification in very mild conditions with a high reaction efficiency[198–202]. Some lipid or hydrophobic molecules can insert into the membrane structure for anchoring functional groups on the surface of nanovesicles easily[203,204]. Besides, a method similar to the preparation procedure that includes co-incubation, sonication, incubation with membrane permeabilizers, freeze and thaw cycles, electroporation and extrusion, can be adopted to encapsulate different kinds of molecules into the core of PDNVs[205,206]. Moreover, the functions of different kinds of membrane-based vesicles can be integrated *via* membrane fusion, so that the mixed structure would inherit the properties from their distinct parental cells[207–209]. It should be noted that these post-secretion modifications should not compromise the vesicle integrity during the cargo and functionality incorporating process. Additionally, genetic engineering, as a powerful technique to manipulate parental cells, can provide the opportunity to incorporate large biomolecules, such as nucleic acids, proteins, and peptides, into the derived vesicles, rendering the obtained vesicles with desired and functions without the need of post-modification[210,211].

Under physiological conditions, recognition of PDNVs by body defense mechanisms (*e.g.*, RES) must be avoided, as this would reduce the targetability of PDNVs and leads to their rapid clearance from the blood circulation[212,213]. As described above, the PEGylation or PEG-functionalization of PDNVs can be used as a versatile and effective method to lessen their non-specific binding of PDNVs to macrophages and to avoid their clearance by RES[214]. This "stealth" property allows them to have a great EPR effect at the tumor site and a prolonged circulation time[215]. For example, Zhang et al.[160] reported that nanovesicles from *Asparagus cochinchinensis* (ACNVs) with a diameter of about 119 nm significantly inhibited the proliferation of tumors, induced cellular apoptosis by upregulating apoptosis-related factors in hepatocellular carcinoma cells without damaging normal hepatocytes (Fig. 9A). By further modifying PEG on the surface of ACNVs, the resultant PEGylated ACNVs (PEG-ACNVs) showed a significantly improved blood circulation period *in vivo* and an enhanced tumor-targeting ability in tumor sites (Fig. 9B and C). As a result, a significant level of inhibitory efficiency on tumor

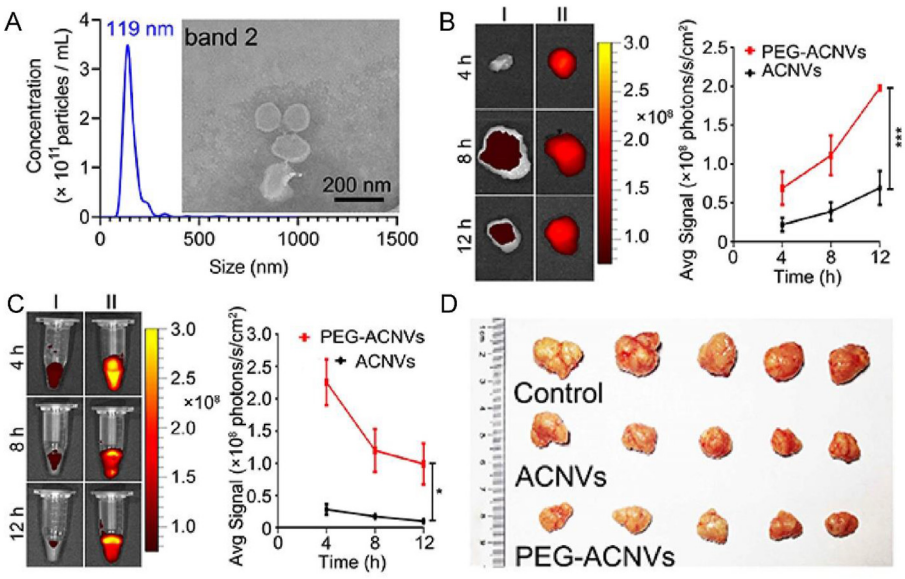

Figure 9 (A) The morphology and size of sucrose-gradient band 2 was characterized by NTA and TEM. (B) *Ex vivo* fluorescence imaging and quantification of tumors from mice treated with ACNVs (I) and PEG-ACNVs (II). (C) *Ex vivo* fluorescence imaging and quantification of blood samples from mice treated with ACNVs (I) and PEG-ACNVs (II). (D) Representative images of tumors in mice after different treatments. Adapted with permission from Ref. 91. Copyright 2021 Dove Medical Press.

cell growth was attained *via* the administration of PEGylated ACNVs without significant side effects (Fig. 9D).

To achieve active targeting ability, Xiao et al.[61] designed a bionic drug delivery system called HRED using extracellular vesicles from lemons. It was fabricated by functionalizing heparin-cRGD (HR) on the EV surface, then loading it with doxorubicin (DOX). The introduction of HR allowed HRED to increase the intracellular ROS and subsequently decrease the production of ATP through enhanced endocytosis and micropinocytosis. Although HRED barely down-regulated P-gp expression, it effectively inhibited the efflux function of P-gp, thus efficiently overcoming the multidrug resistance of DOX-resistant ovarian cancer. In another study reported by Wang et al.[154], it was shown that nanoparticles made of GNVs were demonstrated to actively deliver proteins, siRNA, DNA expression vectors, and chemotherapeutic drugs into several types of cells *via* the assistance of tumor targeting moiety (Fig. 10A and B). They combined GNVs with folic acid (FA) and studied the efficacy of nanoparticles on anticancer therapy. The FA-modified GNVs showed an enhanced targeting efficacy on tumors in a physiological milieu, as demonstrated by the inhibited effects of FA-modified and PTX-loaded GNVs on implanted tumor growth (Fig. 10C and D). The results also demonstrated that GNVs can be loaded with hydrophobic agents including curcumin, FA, and Zymosan A without affecting their biological functions. For example, cells treated with GNVs carrying biotinylated eYFP DNA expression vector expressed the same level of YFP protein as compared with the cells transfected with Lipofectamine 2000. Also, the transfection efficiency of splenocyte CD4 or CD8 T cells as well as the expression of the luciferase gene are both considerably increased by GNVs loaded with corresponding biotinylated proteins (*i.e.*, anti-CD4 or anti-CD8 antibodies). They also found that GNVs could improve the delivery efficiency of siRNA into tumor cells.

In their subsequent study, GNVs were coated with the activated leukocytes-derived cell membrane that was enriched with inflammatory related receptor (IGNVs) and had no significant changes in particle size (Fig. 11A)[155]. IGNVs highly express chemokine receptors and integrins, while inflammatory tissues have high concentrations of chemokines, so the transmigration capability of endothelial cells is significantly enhanced, thereby promoting the accumulation of IGNVs in inflammatory tissues (Fig. 11B). They further showed that intravenous injection of IGNVs loaded with either DOX or anti-inflammatory agent (*i.e.*, curcumin) could significantly inhibit the growth of colon and breast cancer cells and attenuate colitis caused by DSS, respectively. Furthermore, they developed a GNV-based nanovector hybrid with polyethylenimine (pGNV), which was subsequently modified with FA (FA-pGNVs) to enhance the targeting efficacy[156]. MiR17-loaded FA-pGNVs were administered intranasally to quickly transport the encapsulated genes to the brain, which were selectively uptaken by brain GL-26 tumor cells and inhibited GL26 tumor growth. What's more, Zhang et al.[158] showed that nanovectors made of ginger-derived nano-lipids (GDNVs) exhibited an excellent biocompatibility and had a high loading efficiency for[158]. The resultant nanoplatform showed a better performance than the commercially available liposomal-DOX in pH-dependent drug release. Their experiments also showed that DOX-loaded FA-modified GDNVs significantly suppressed the tumor growth in the Colon-26 mice model (Fig. 12A). Alternatively, Li et al.[159] used ultracentrifugation combined with density gradient centrifugation to isolate nanovesicles from ginger roots (Fig. 12B). Ligand-displaying arrowtail RNAs was used to engineer these nanovesicles and FA was chosen as a targeting ligand to conjugate with arrowtail RNA (Fig. 12C and D). The results suggested that such approach could successfully silence the survivin gene expression and suppress tumor growth in epithelial cancer models *via* intravenous administration of survivin siRNA (Fig. 12E).

4. Clinical trials of PDNVs related to cancer

As mentioned above, due to the advantages of PDNVs in cancer therapy, several clinical trials are underway or have been

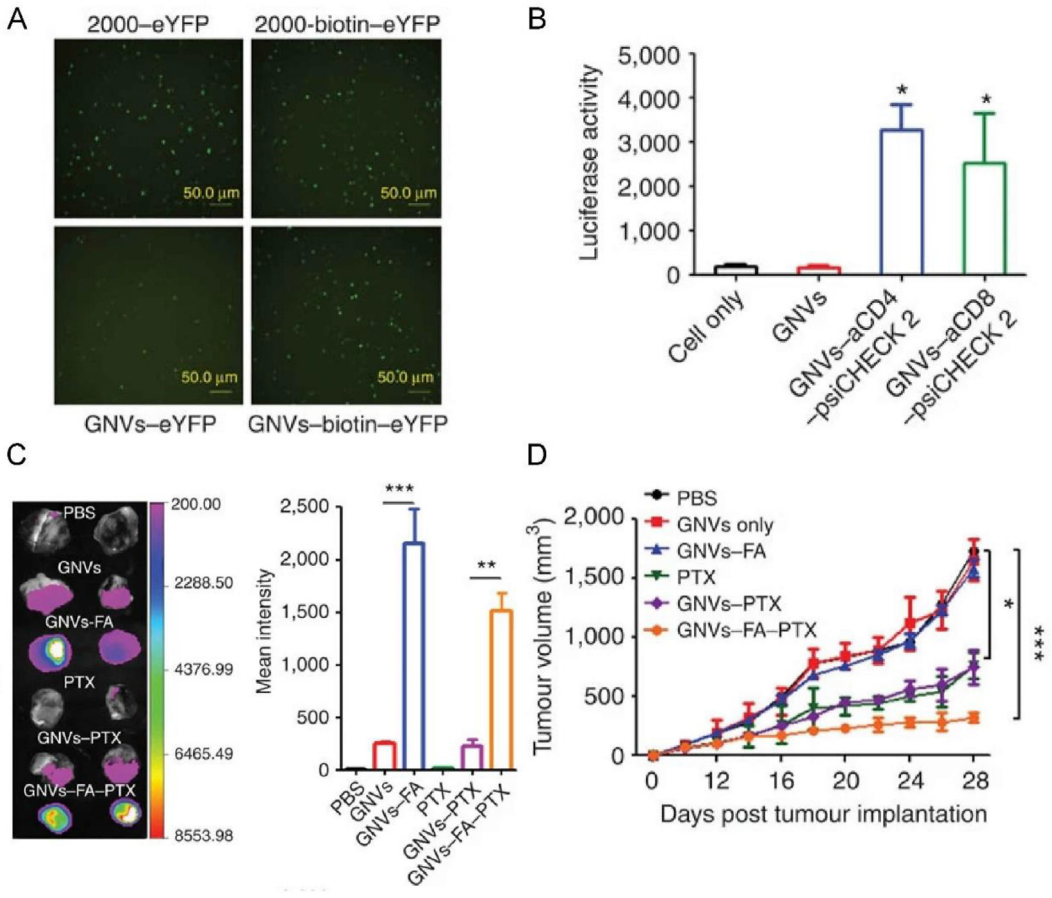

Figure 10 (A) Representative images of cells transfected with biotinylated eYFP using GNVs or Lipofectamine 2000. (B) Activity of luciferase expressed in T cells transfected with biotin-labeled anti-CD4 or CD8 antibodies loaded with GNVs encapsulated with psiCHECK2. (C) Representative images of each group of tumors after treatment (left) and mean intensity of fluorescence signals (right). (D) Changes of tumor volume in different groups after subcutaneous injection of tumor cells. Adapted with permission from Ref. 93. Copyright 2013 Nature Publishing Group.

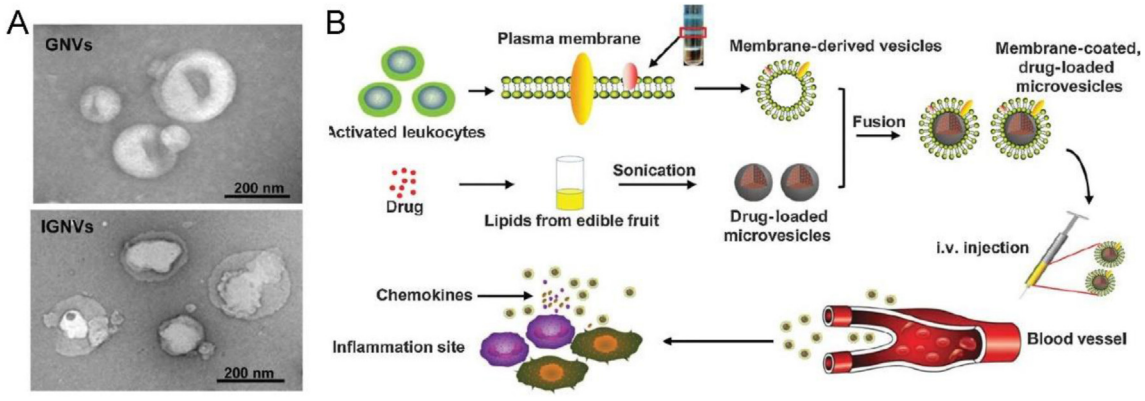

Figure 11 (A) Scanning electron microscopy image of GNVs and IGNVs. Adapted with permission. (B) Schematic of the preparation of the IGNVs and GNVs for targeted delivery of drugs to the sites of inflammation.[9] Adapted with permission from Ref. 94. Copyright © 2015 American Association for Cancer Research.

completed. Zhang et al.[122] found that GDNVs can reduce acute colitis and prevent chronic colitis and CAC. They, then, conducted clinical trials to evaluate whether GDNVs or curcumin alone or combined with curcumin had clinically important anti-inflammatory effects on the intestinal lining of IBD patients, but no studies related to the prevention of CAC were conducted (NCT04879810). The trial has been completed, but the research results have not yet been published. Dryden GW et al. plan to address the problem of drug delivery by using PDNVs to more effectively deliver curcumin to colon tumors and normal colon

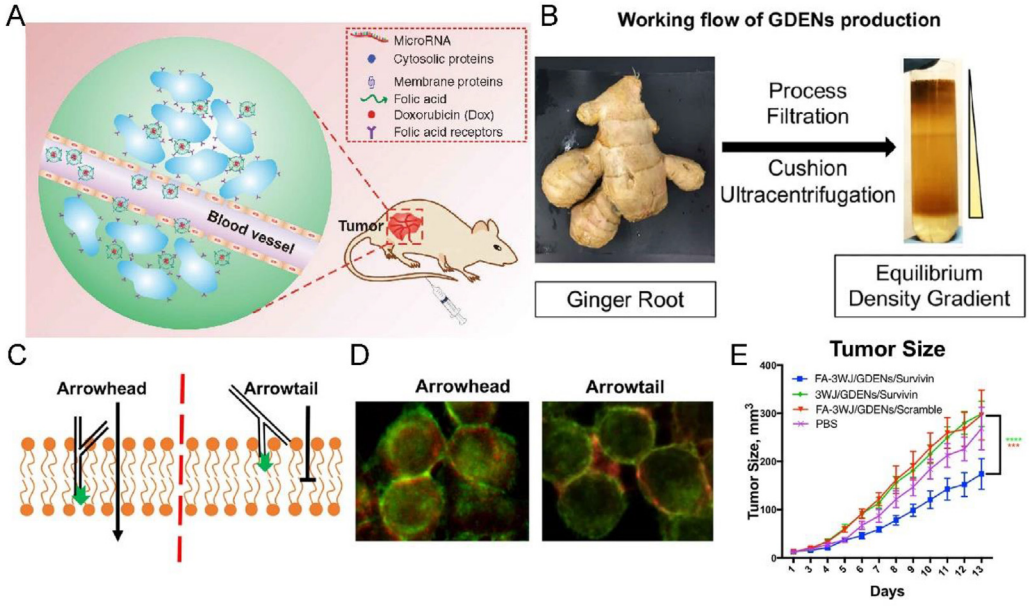

Figure 12 (A) Schematic of intravenous injection of GDNVs for targeted delivery of chemotherapeutic agents to tumors through blood vessels. Adapted with permission. Adapted with permission from Ref. 96. Copyright © 2016 Elsevier. (B) Process for isolating nanovesicles from ginger roots (GDENs). (C) Concept of ligand displaying by arrowtail and partially loading by arrowhead due to different orientation. (D) Fluorescence microscope image of Alexa647-labeled arrowtail and arrowhead RNA nanoparticle (red) incubated with KB cell (green). (E) Size of KB cell-derived xenograft tumors in nude mice treated intravenously every two days for two weeks. Adapted with permission from Ref. 97. Copyright 2018 Nature Publishing Group.

tissue (NCT01294072). This study did not complete patient recruitment. Another clinical trial aimed to evaluate the ability of grape nanovesicles as an important anti-inflammatory agent to reduce the incidence of oral mucositis during radiotherapy and chemotherapy for head and neck tumors (NCT01668849). This clinical trial has completed phase 1.

Even though PDNVs offer tremendous advantages in cancer therapy, their transition from scientific research, clinical trials to true clinical application has not yet been achieved. None of the above three clinical trials has yet available study results for reference, whether uncompleted or completed. Also, according to their publications, the methods used to isolate PDNVs may still be laboratory methods, including ultracentrifugation, which means that safe, pure, and efficient consistent production outside the laboratory has not yet been achieved[122,216]. Good manufacturing practices (GMP) guidelines need to be established as soon as possible to regulate the isolation, production, downstream purification and analysis of PDNVs[217]. In addition, there have been no clinical trials of PDNVs in combination with clinical anticancer drugs. Nanomedicines such as liposomal doxorubicin have been approved for the treatment of various cancers and have demonstrated significant antitumor activity in clinical practice, signaling a possible application to replace conventional anticancer drugs in treatments such as chemotherapy utilizing the unique pharmacology of PDNVs in combination with chemotherapeutic drugs[218].

5. Current challenges and future perspectives

Over the last few years, research on the function of PDNVs in biological systems and their activity in the treatment of disease has attracted great interest. In this review, we summarized the current findings and advances in this field and clearly demonstrated that certain types of PDNVs had a potential of anticancer capacity and might function as drug vectors to enhance the effectiveness of anticancer therapeutics. The diversity and abundance of PDNVs carrying lipids, proteins, nucleic acids, and other bioactive molecules could provide more therapeutic options than traditional synthetic materials and free drugs[219–221].

Despite the significant advancements in this newly burgeoning field, there are still many challenges for the exploration of PDNVs as innovative therapeutics. As a naturally obtained product from fresh vegetables or fruits, the isolation method for PDNVs should be further improved, since the current approaches usually need repeated centrifugation, which may result in large amount of product loss and increase the cost of time and expenditure for obtaining desired PDNVs.

In addition, current approaches usually generate nanovesicles with heterogeneous structures including different sizes and/or structures, giving rise to the obtained products with multiple impurities. To this end, advanced techniques should be applied to confirm the quality, stability, and purity of isolated PDNVs with a large-scale production. Also, the chemical compositions of plants are sensitive to multiple factors, which have a significant impact on their biological effects. PDNVs isolated from plants in different environments, seasons, and even different parts of plants can hardly be considered to have exactly the same chemical compositions. In light of this, even through numerous efforts have been made for the isolation and purification of PDNVs, it is still necessary to control various conditions in the production of PDNVs, including the plant growth environment, plant processing means and PDNVs isolation process, to ensure that the final PDNVs have a qualified quality. However, up to now, a standardized, GMP-compliant method is still missing.

Furthermore, long-term preservation is also critically important for PDNVs' application. The intactness of the membrane structure and biological function of PDNVs-associated substances should be kept intact and stable, especially after incorporating additives. Compared with the commercial methods like dehydration, rapid freezing is regarded as a viable choice for PDNVs' preservation, and for long-term storage, −80 °C is recommended to prevent the degradation of active compounds (*e.g.*, proteins and nucleic acids)[222−224].

Most importantly, our comprehension of the interaction between PDNVs and cancer cells and how the various bioactive molecules contained in PDNVs enter the cells and exert their corresponding effects is still limited, especially when different modes of administration are used. For example, a team recently tried to use tea flower- and tea leaf-derived nanovesicles to treat breast tumors[60,118]. They found that PDNVs, whether administered intravenously or orally, made significant antitumor effects in mouse breast cancer models. Although the accumulation of PDNVs at the tumor is weaker than intravenous administration with oral administration, they caused no detectable toxicity to healthy organs when used in high doses. In contrast, high-dose intravenous PDNVs could stimulate immune system activation, induce liver and kidney toxicity, and change hemogram. Other literature on the intravenous use of PDNVs didn't report this phenomenon. For biomedical applications of cancer therapy, the most common method of administration is intravenous since oral administration usually needs the drugs to resist the harsh gastrointestinal environment and cross biological barriers, which make the development of oral-administered drug delivery systems challenging[225,226].

In nature, there are numerous species of plants that are available to prepare nanovesicles containing bioactive substances, many of which have great therapeutic importance for treating disease. However, their biological functions in living systems have not been well understood so far, since these naturally occurring architectures are much more complicated than simple lipidic vesicles. Correspondingly, vital concerns, including how do they affect health and disease development, which substance is critical for eliciting pharmacological properties, how do they participate in cellular communication, and how do they modulate tissue or cell's function, require more efforts to be conducted for biomedical use. Systematic study of their interaction with biological systems and their toxicity and safety *via* long-term *in vivo* evaluation would be more valuable to verify the therapeutic potential of PDNVs-based plarforms for practical use. Moreover, diverse kinds of PDNVs possess different components with particular biological functions. For task-specific application, different nanovesicles from new kinds of plants should be continuously developed, and information on their role in influence on cancer progression should be carefully evaluated, which are allowing new material designs to expand the biomaterials toolbox for treating cancer.

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Author contributions

Hanzhe Liu wrote the initial draft of the review. Guo-Feng Luo and Zhengjun Shang revised the manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

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