Herbal Medicine-Derived Exosome-Like Nanovesicles: A Rising Star in Cancer Therapy

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Abstract: Plant-derived exosome-like nanovesicles (PDNVs) are small nanoscale vesicles containing lipids, RNAs, proteins and some plant natural products secreted by plant cells. Over the last decade, PDNVs have garnered significant interest due to its exceptional therapeutic benefits in the treatment of various diseases. Herbal medicine, as a medicinal plant, plays an important role in the treatment of diseases including cancer. Especially in recent years, the function of herbal medicine derived exosome-like nanovesicles (HMDNVs) in the treatment of cancer has been widely concerned, and has become a research hotspot of nanomedicine. In this review, the biological characteristics, functions and the therapeutic advantages of PDNVs are reviewed, as well as the recent achievements and research progress of HMDNVs in cancer treatment, demonstrating its enormous promise as a cancer therapy, and new insights are provided for future research and development of anti-tumor drugs.

Keywords: herbal medicine, plant-derived exosome-like nanovesicles, extracellular vesicles, cancer treatment, nanomedicine

Introduction

Cancer ranks a leading cause of premature death in most countries around the world,[1] its morbidity and mortality rates are still increasing rapidly. According to data from GLOBOCAN, in 2022, there were approximately 20 million new cancer cases and nearly 9.7 million deaths worldwide, and in 2050, there will be an estimated 35 million new cancer cases worldwide.[2] As the most important and effective methods for cancer treatment before, radiotherapy and chemotherapy have played a great role in improving the survival rate and quality of life of cancer patients, but they also show great toxicity,[3,4] specifically, they not only inhibit or kill rapidly proliferating cancer cells, but also act on rapidly proliferating normal cells, such as bone marrow, hair follicle and gastrointestinal tract cells. The aforementioned strategy, which lacked specificity and was suboptimal, underwent a transformation upon the discovery of cell signaling networks implicated in the regulation of cell proliferation and differentiation. This breakthrough facilitated the development of drugs that selectively target these networks, thereby paving the way for the future implementation of targeted therapy and immunotherapy (Figure 1),[5] which aim to inhibit the signaling pathways that facilitate uncontrolled proliferation of malignant cells, or precisely deliver chemotherapy agents and biological antibodies exclusively to cancer cells, thereby minimizing harm to normal cells and mitigating undesirable side effects.[6,7]

Over the past few decades, with plant natural products extracted from herbal medicines showing safety and efficacy in anticancer activity, holding the promise for improving chemotherapy and being a new drug for cancer treatment, it has attracted wide attention.[8] However, it still suffers some limitations, such as poor solubility, low bioavailability, short half-life, etc.[9] Although the synthetic nanoparticles and liposomes coated with active substances have been developed to solve these problems well, yielded notable reductions in negative health consequences and improved delivery efficiency due to the enhanced permeability and retention effect (EPR)
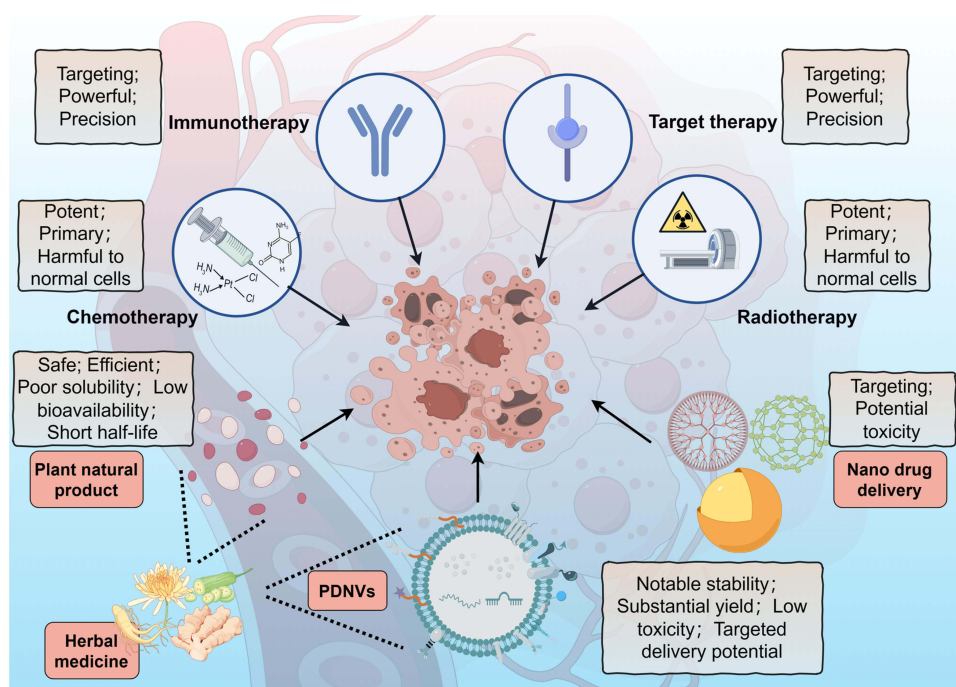
Figure 1 Methods of cancer treatment. Chemotherapy and radiotherapy have traditionally been the primary modalities in cancer treatment, however, their non-specific effects on normal cells have prompted the development of more precise and targeted immunotherapies and targeted therapies. In addition, plant natural products have shown promise in cancer treatment due to their anti-cancer properties. To address challenges such as poor solubility and short half-life associated with these natural products, nano delivery methods utilizing metal nanoparticles and artificial liposomes have been developed, but also raises the question of potential toxicity. PDNVs have inherited the anti-cancer activity of natural products combined with plant natural nanovesicles, which have great potential in cancer treatment. The figure was drawn by Figdraw.

meanwhile,[10,11] the issues pertaining to premature drug release during the synthesis, storage, or circulation of nanoparticles, as well as concerns regarding potential toxicity and the absence of tumor specificity, also emerged (Figure 1).[12] As a result, the number of drug-loaded nanoparticles that have received approval for clinical applications remains limited.[13]

However, PDNVs, plant-derived extracellular vesicles resembling exosomes and characterized by a phospholipid bilayer enclosing RNA, proteins, lipids, and various bioactive compounds, have recently garnered attention as a potential therapeutic approach in the field of oncology owing to their notable stability, substantial yield, low toxicity, targeted delivery potential, and effectiveness (Figure 1). These PDNVs not only capitalize on the advantages of artificial nanoparticles while circumventing their drawbacks, but also exhibit substantial potential and application value in the field of cancer treatment. Moreover, within the PDNVs family, HMDNVs, which inherit therapeutic properties from their herbal plant origins, demonstrate enhanced tumor destruction capabilities, are anticipated to emerge as a new star in cancer treatment.

PDNVs
Definition of Exosomes

Eukaryotic cells secrete three main types of extracellular vesicles (EVs), each has a unique biogenetic mechanism. The largest of these are known as apoptotic bodies (1000–5000 nm in diameter), which are membrane vesicles released from cells during late programmed cell death. The other two classes of EVs are microvesicles (100–1000 nm in diameter) that bud off directly from the plasma membrane and exosomes (30–150 nm in diameter) that are released from cells after multivesicular bodies (MVBs) fuse with the plasma membrane (Figure 2).[14,15] Among them, exosomes are nanovesicles with a phospholipid bilayer structure, containing a variety of proteins, lipids and RNAs that play an important role in intercellular communication.[16,17]
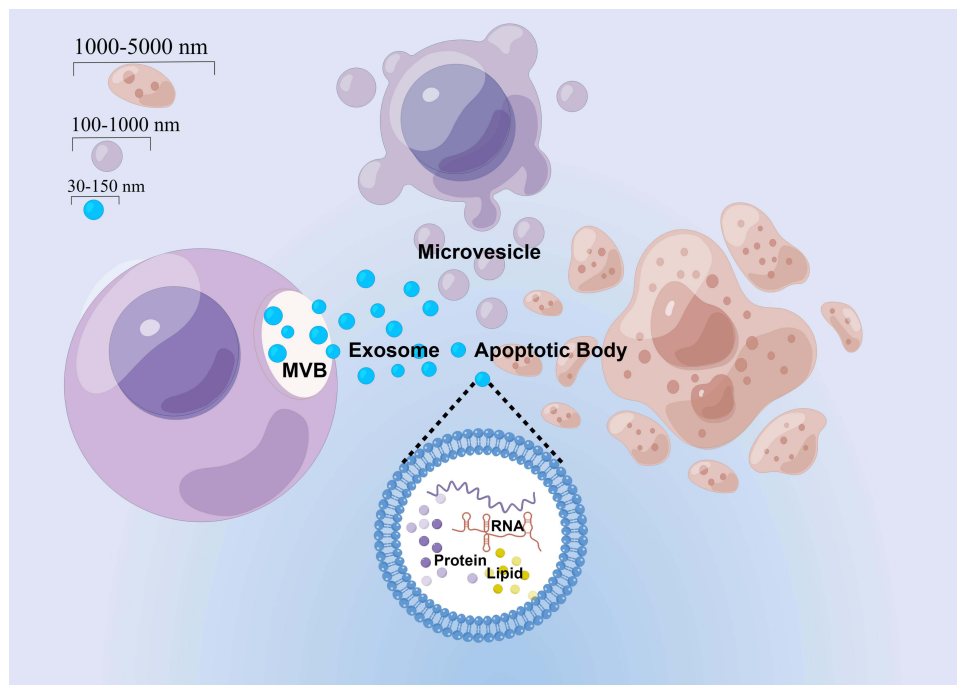
Figure 2 Three main types of EVs secreted by eukaryotic cells. Three main types of EVs include apoptotic bodies (1000–5000 nm in diameter) produced by cells in apoptosis as well as microvesicles (100–1000 nm in diameter) and exosomes (30–150 nm in diameter) secreted by cells. The figure was drawn by Figdraw.

The Origin of Exosomes

In 1983, cell release vesicles were first observed during in vitro culture of sheep reticulocytes, which could be harvested by centrifugation at 100000g for 90 min,[18] and were confirmed and officially referred to as exosomes more than a decade later.[19] Since then, mammalian exosomes have ushered in a research peak. In addition, in 2007, it was found that exosomes contain mRNA and miRNA, which can be transferred to another cell and function in this new location. The research on exosomes was further pushed to a climax.[20]

Plant cells, unlike mammalian cells, have cell walls that may inhibit the creation and release of EVs, hence research into plant exosomes has been put on hold. However, there is growing evidence that plants also secrete similar exosome-like vesicles. Even 16 years earlier than the first mammalian exosomes were observed, as early as 1967, carrot MVBs have been observed to fuse with the plasma membrane and secrete similar exosome-like vesicles outside the cell.[21] It was not until 2009 that plant exosomes were first isolated from sunflower seeds by Regente et al.[22] Since then, PDNVs have gradually begun to be studied. In recent years, PDNVs have been isolated from fruits, roots, seeds, and leaves of various plants by standardized exosome separation and purification techniques such as ultracentrifugation, and their physico-chemical properties (size, shape, surface charge, contents, etc.) and therapeutic activities in different diseases have been extensively studied.[23]

Physiological Function of PDNVs

Initially, it was thought that exosomes were produced by cells as a way of getting rid of substances they did not need.[24] Yet, there is mounting evidence that exosomes play a critical role in cellular communication, mammalian reproduction and development, immune response and infection, and the incidence of a wide range of illnesses.[25] In a similar vein, growing research on plant exosomes has revealed that these tiny particles are crucial for sustaining immune defense, intercellular communication, and cell homeostasis (Figure 3).

Maintain Cell Homeostasis

After being attacked by the pathogen, MVB starts to multiply rapidly in infected plant cells and releases exosomes more often. These exosomes, which contain a lot of hydrogen peroxide and callose, build up surrounding the nearby healthy
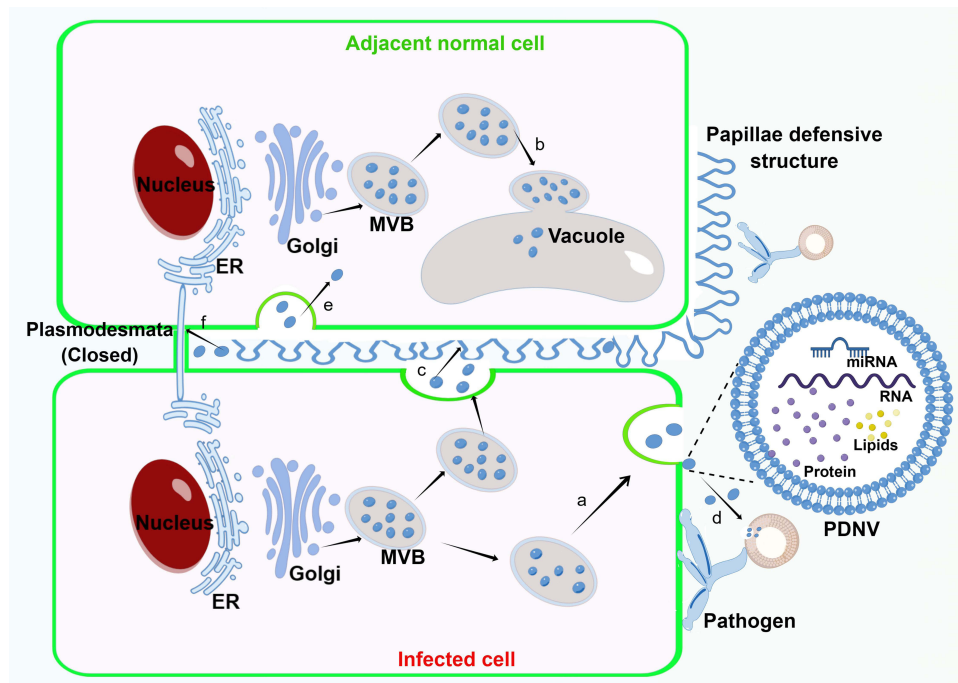
Figure 3 Biogenesis and physiological function of PDNVs. a. Fusion of MVB with the plasma membrane results in the release of PDNVs. b. Fusion of MVB with vacuolar or lysosomal membranes results in degradation of the contents. c. PDNVs with large amounts of hydrogen peroxide and callose accumulate around adjacent normal cell to form papillae defensive structures against pathogen invasion. d. PDNVs are taken up by pathogens and silenced its genes critical to pathogenicity. e. PDNVs are taken up by adjacent cells to maintain intercellular communication. f. PDNVs block plasmodesmata for preventing the further penetration of pathogen and the propagation of apoptotic signals. The figure was drawn by Figdraw.

cells, producing protective papillae structures. At the same time, they close off the plasmodesmata, thus preventing the further penetration of fungi and the propagation of apoptotic signals, and finally maintain cell homeostasis.[26]

Immune Defense

Cai et al discovered that *Arabidopsis* cells secrete exosome-like extracellular vesicles capable of delivering sRNAs to the fungus *Botrytis cinerea Pers*. These vesicles containing sRNAs congregate at the site of infection and are taken up by fungal cells to silence fungal genes critical to pathogenicity.[27]

This suggests that *Arabidopsis thaliana* has evolved to use exosome mediated cross-kingdom RNAi as a part of its immune response in the arms race against pathogens for a long time.

In addition, plants can rapidly transport intracellular signal peptide-deficient proteins to the cell extranet to combat biological and abiotic stresses via an exosome pathway independent of the classical endoplasmic reticulum - Golgi secretory pathway. On average, the majority of these signal peptide-deficient proteins, which make up about 50% of the secreted proteins, have antibacterial activity.[28]

Intercellular Communication

Furthermore, several proteins involved in immune signaling were also identified in an analysis of the proteomics of pathogen-infected *Arabidopsis* exosomes, suggesting that, similar to mammalian exosomes, plant exosomes regulate pathogen recognition by facilitating extracellular transport of key signaling proteins, thus transmitting signals to neighboring cells for enhanced pathogen detection.[29]

Possible Biogenetic Pathway of PDNVs

The release of exosomes by MVB fusion with the plasma membrane is considered to be the main pathway of exosome production in mammalian cells,[30] while MVB-mediated exosome-like vesicles secretion has also been observed in different plant cells of different plant species by transmission electron microscopy (TEM).[31] Therefore, it is thought that the same biogenetic pathway may exist in plants. MVBs are late endosomes (LE) containing intraluminal vesicles (ILVs)

that are produced by limiting membrane invagination and budding into the lumen. As with mammalian cells, ESCRT complexes (ESCRT-0, I, II, and III) are thought to be involved in the maturation of ILVs and cargo sorting of ILVs in plants. Although the plant has no canonical ESCRT-0 subunit, it contains ubiquitin-bound TOM1-like (TOL) proteins instead. The formation of ESCRT-I and II complexes deforms the membrane into a bud, which then recruits ubiquitin-binding protein TOL, allowing cargo to be transported into the bud. Finally, the ESCRT-III subunit is recruited by the ESCRT-I and II complexes to the bud neck, contract the plasma membrane and cut the bud to form an ILV. Eventually, MVB fuses with the vacuole or lysosome membrane leading to degradation of its contents or fuses with the plasma membrane leading to its release in the form of exosomes rather than ILVs (Figure 3).[32,33]

In addition, a recent study found that proteins enriched in *Catharanthus roseus* (L). Don leaves-derived PDNVs contained MVB and ILV associated proteins involved in late endosome maturation or MVB-mediated vesicular trafficking, which provided evidence for the biological origin of the MVB pathway in PDNVs.[34]

The Contents of PDNVs and Their Functions

PDNVs were found to contain mainly lipids, proteins, RNA, and some plant natural products (Figure 4).[35,36]

Lipids

The presence of a lipid bilayer structure in PDNVs is crucial for their transport capability and the stability of their contents.[37] Therefore, lipids play a very important biological role in PDNVs. Teng et al showed that the exosomal membranes of PDNVs are enriched with phosphatidic acid (PA), phosphatidylcholines (PC), phosphatidylethanolamine (PE), digalactosyldiacylglycerol (DGDG), and monogalactosyldiacylglycerol (MGDG). However, PDNVs sourced from different origins exhibit variations in lipid composition. For instance, ginger and turmeric PDNVs-derived lipids are enriched with PAs, comprising 35.2% and 34.4% of the total lipid content, respectively. In contrast, grapefruit and garlic PDNVs contain significantly lower levels of PAs, representing only 3.5% and 5.5% of
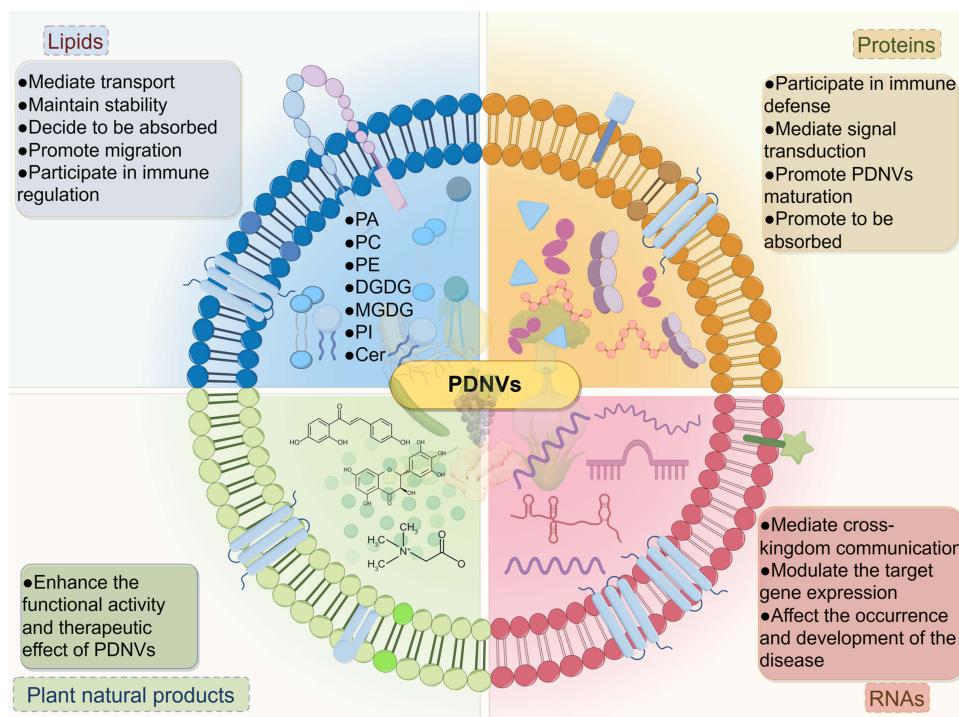

Figure 4 The contents of PDNVs and their functions. The contents of PDNVs primarily consist of lipids, proteins, RNAs, and plant natural products, each serving a crucial function. Lipids are primarily responsible for maintaining the stability and facilitating transport within PDNVs, proteins play a role in immune defense and signal transduction, RNAs mediate cross-kingdom communication and regulate gene expression, and plant natural products contribute to the overall activity of PDNVs. The figure was drawn by Figdraw.

the total lipid content, respectively. Instead, the predominant lipid species in grapefruit and garlic PDNVs is PC, accounting for 36.2% and 52.6% of the total lipid content, respectively. Furthermore, it was observed that grape-derived PDNVs exhibited a high percentage of PA at 53.2% and PE at 26.1%.[37] On the contrary, PDNVs derived from oranges juice contained PE,~40%, PC,~25%, phosphatidylinositol (PI,~12%) and PA,~5%,[38] which closely resemble the lipid composition of grapefruit-derived PDNVs; in particular, their PE/PA ratio is opposite to that of grapefruit- or ginger-derived PDNVs. These results suggest that Rutaceae plant-derived PDNVs have a similar specific lipid composition.

These distinct lipid ratios found in PDNVs contribute to their specific functions. Teng et al have demonstrated that PDNVs derived from edible plants exhibit a preference for uptake by gut bacteria in a manner dependent on the lipid composition of PDNVs. Specifically, PDNVs enriched with PC lipids, such as those derived from grapefruit, are preferentially taken up by *Ruminococcaceae sp*. Conversely, PDNVs enriched with PA lipids, such as those derived from ginger, are preferentially taken up by *Lactobacillaceae*. Furthermore, PA lipids are involved in regulating the duration and quantity of PDNVs accumulation in the gut, PC lipids facilitate the migration of PDNVs from the intestine to the liver.[39] Another study revealed that the unsaturated state of PA lipids plays a crucial role in the uptake of ginger-derived PDNVs by pathogens, with PA (34:1) not absorbed by *Porphyromonas gingivalis* compared to PA (34:2).[40] In addition, the substantial presence of ether phospholipids in Catharanthus roseus-derived PDNVs potentially accounts for its exceptional stability and facilitates its involvement in the immunomodulatory mechanisms of host cells.[34] The binding of Garlic-derived PDNVs PA (36:4) to microglial cell Brain Acid-Soluble Protein 1 (BASP1) is necessary for the internalization of Garlic-derived PDNVs and the subsequent suppression of indoleamine 2,3-dioxygenase 1 (IDO1) expression.[41] Ginseng-derived PDNVs were enriched with DGMG, 59.4%, PE, 16.8% and ceramide (Cer, 13.8%). Different from other PDNVs, this high content of DGMG and unique ceramide may be involved in changing the polarization of macrophages (Figure 4).[42]

Proteins

Proteins play a crucial role in the execution of gene function and significantly contribute to the functioning of PDNVs. Numerous studies have documented the enrichment of proteins within diverse PDNVs. For instance, a proteomic analysis conducted on *Arabidopsis* EVs revealed a notable enrichment of proteins associated with biotic and abiotic stress. Particularly, these proteins were involved in signaling and immune responses, indicating a potential specialization of these EVs in immune defense and stress adaptation. Furthermore, the presence of proteins associated with reactive oxygen species (ROS) signaling and membrane transport proteins within EVs suggests their potential role in regulating ROS levels, promoting ROS signaling, and facilitating EVs formation and secretion.[28] In a study conducted by Ou et al, a comprehensive analysis of 1843 proteins in *Catharanthus roseus* (L). Don leaves-derived PDNVs was performed, revealing 1184 differentially expressed proteins compared to the plant proteome. Notably, 471 significantly up-regulated proteins, primarily encompassing transporters and signaling proteins, were identified, potentially contributing to the maturation of *Catharanthus roseus* (L). Don leaves-derived PDNVs and signaling processes.[34]

Furthermore, the researchers conducted an experiment wherein M2-like macrophages were treated with PDNVs derived from ginseng that had been digested with DNase I/RNase I. The results indicated that there were no significant differences in the induction of IL-6 and TNF-α compared to the undigested group, and there was no effect on the upregulation of surface markers in M1-like macrophages. Conversely, in the proteinase K digestion treatment group, only a minimal amount of IL-6 and TNF-α was induced, and the upregulation of M1-like macrophage surface markers did not occur. These findings suggest that protein, rather than nucleic acid, is involved in the polarization of macrophages by ginseng PDNVs.[42] Similarly, after the elimination of the surface proteins of garlic-derived PDNVs through trypsin digestion, the group subjected to digestion exhibited a reduced level of cellular uptake in comparison to the undigested group. This finding suggests the significant involvement of surface proteins in the internalization process. Subsequent investigations revealed that the mannose-specific binding protein, II lectin, located on the membrane surface of garlic-derived PDNVs, interacted with cluster of differentiation 98 (CD98) on the cell membrane surface, thereby facilitating their uptake by cells (Figure 4).[43]

RNAs

Microribonucleic acid (miRNAs) is a class of small (18–24 nt) non-coding RNA that plays an important role in post-transcriptional gene regulation in animals and plants.[44] Studies have shown that exogenous plant miRNAs are present in the serum and tissues of a variety of animals, that these exogenous plant miRNAs are mainly obtained through food intake, and that the possible carriers of these plant-derived miRNAs are plant extracellular vesicles.[45]

In fact, several studies have demonstrated the presence of RNA, particularly miRNA, in PDNVs. These miRNAs can be assimilated by host cells and subsequently modulate the expression of target genes, which greatly supports the cross-kingdom regulation of plant-derived miRNAs. For example, a deep sequencing of ginger derived PDNVs by Zhang et al revealed 125 different miRNAs in them, ranging in length from 15 to 27 nucleotides, binding software analysis showed that 124 of these microRNAs may target and regulate human gene expression by binding to their 3'-untranslated regions (3'-UTRs).[35] Moreover, Xiao et al further analyzed the miRNA profile of 11 PDNVs derived from different fruits and vegetables and showed that some of the highly expressed miRNAs could regulate the expression of inflammatory cytokines and cancer-related genes in vitro.[46] These results suggest that PDNVs-derived miRNAs can also mediate cross-kingdom communication, regulate the expression of related genes in the recipient host cells, and affect the occurrence and development of a variety of diseases (Figure 4).

Plant Natural Products

With the advancement of research on plant natural products and the enhancement of extraction technology, a new era has emerged in the field of utilizing plant natural products for the prevention and treatment of complex diseases.[47] For instance, it has been observed that approximately 49% of cancer drugs approved by the food and drug administration (FDA) between the 1940s and 2014 were either plant natural products or derived directly from them.[48] The accumulating evidence indicates that PDNVs not only possess certain characteristics inherited from their source plants but also contain plant natural products that hold potential therapeutic value. For instance, PDNVs derived from cucumbers possess bioactive secondary metabolites, notably cucurbitonin B, which has been documented to impede the progression of leukemia, breast cancer, lung cancer, and liver cancer;[49,50] Furthermore, the presence of sulforaphane compounds in broccoli-derived PDNVs has been found to hinder the development of diverse cancers, such as pancreatic, intestinal, leukemia, and prostate cancers. The manipulation of sulforaphane through knock-out and knock-in experiments has demonstrated its significant association with the biological effects of PDNVs.[51,52] A separate study conducted an isolation of three distinct sizes of ginger nanovesicles, revealing that 6-gingerol and 6-shogaol are inherent active constituents common among them. Furthermore, their findings demonstrated that PDNVs with elevated concentrations of natural products (5.68 μg/mg 6-gingerol and 2.95 μg/mg 6-shogaol) exhibited a more pronounced therapeutic impact, thereby indicating the significance of natural products in the therapeutic efficacy of ginger-derived PDNVs (Figure 4).[35]

The intricate and heterogeneous lipids, RNAs, proteins, and natural products found in PDNVs are essential for cellular communication and intercellular material exchange, which may also make PDNVs promising candidates for the treatment of human diseases, especially cancer. Consequently, comprehensive knowledge regarding the constituents and potential functions of each PDNV is crucial for the advancement of novel drugs derived from PDNVs.

Advantages of PDNVs as Therapeutic Agents

PDNVs display a number of clinical characteristics and exceptional therapeutic advantages over mammalian exosomes or synthetic nanoparticles, including ease of mass production, low immunogenicity and toxicity, effective cellular uptake, and excellent stability (Figure 1).

Easy to Mass Produce

The yield of the drug itself is a prerequisite for its large-scale use, and PDNVs come from the plant itself, which is exactly what it is. Li et al showed that it was possible to process up to 3 liters of ginger juice in 1 hour, equivalent to 300 cell petri dishes (150 mm), with a yield ratio 300 times higher than the production of mammalian exosomes, just by using ordinary juicers.[53]

Low Toxicity and Immunogenicity

In order to realize the potential of PDNVs in nanomedicine, full attention needs to be paid to safety and toxicology issues. Unlike artificial nanoparticles and liposomes, which contain potential toxicity in vivo, or exosomes secreted by mammalian cells, which contain immunogenicity introduced by secretory cells,[54,55] PDNVs come mostly from edible plants, so they are low toxic to humans. To support this, in a safety test of cucumber sarcocarp-derived PDNVs by Chen et al, hemolysis tests showed that intravenous these PDNVs did not cause erythrocyte rupture, nor did it cause an increase in serum levels of pro-inflammatory cytokines (including TNF-α and IL-1β) in mice after 7 days of caudal intravenous cucumber sarcocarp-derived PDNVs; In addition, HE staining showed no obvious damage to myocardial, liver, spleen, kidney and other organs of these PDNVs treated mice. These results indicated that cucumber sarcocarp-derived PDNVs was non-toxic and safe in vivo and in vitro.[50] What is more, unlike artificial nanoparticles that cross the placental barrier in pregnant mice and cause pregnancy complications, Wang et al injected DiR labeled Ginger-derived PDNVs into the tail vein of pregnant mice and found that these PDNVs did not cross the placental barrier, suggesting that Ginger-derived PDNVs is also safe for the fetus in pregnant women and can be used as delivery vectors for certain drugs in pregnant women.[56]

Efficient Cellular Uptake

Efficient uptake of nanoparticles by target cells is critical for their use as drug or intracellular drug delivery vehicles.[57] Therefore, Wang et al, in order to evaluate the efficient uptake of grapefruit derived PDNVs by different cell types, co-cultured different cell types with PKH26-labeled grapefruit derived PDNVs. The results showed that most cells such as GL26, A549, SW620, CT26 and 4T1 were able to internalize these PDNVs. Moreover, more than 20% of B cells and 14% of T cells took up these PDNVs within 12 hours of co-culture, which is remarkable and exciting because B cells and T cells are the most difficult to be transfected with any commercially available transfection reagent. In addition, in the cellular uptake efficiency experiment of grapefruit derived PDNVs and cationic liposomes, only about 40% of the cationic liposomes entered the cells. However, more than 80% of grapefruit derived PDNVs were taken up by the cells.[56]

Excellent Stability

In order to ensure patient safety and product efficacy during clinical use, its stability and biocompatibility under the relevant conditions of the target administration route (eg, oral administration, intravenous administration, etc.) are particularly important.[58] Therefore, to investigate the stability of PDNVs in vitro, researchers suspended them in different solutions and judged their stability based on their size change, that is, whether they could still maintain nanoscale size. For example, Zhang et al found that ginger derived PDNVs were very stable in simulated solutions of gastric and intestinal juice and were resistant to freeze/thaw cycles.[35] Similarly, Liu et al found that turmeric derived PDNVs could maintain nanoscale size in simulated solutions of gastric (pH~2) and intestinal (pH~6.5) fluid, demonstrating their stability, indicating that they can be administered orally;[59] Chen et al showed that teaflower derived PDNVs were stable in gastric, small intestine, and colon mock solutions as well as DMEM, indicating that they can be ingested or injected.[60]

Furthermore, the assessment of PDNV stability during extended storage periods and the investigation of optimal storage conditions are crucial for ensuring the reliability of experimental results and the viability of future applications. Hwang et al demonstrated that PDNVs derived from yam remained functionally active after being stored at −80°C for one year, with no significant differences observed compared to freshly prepared samples, which aligns with the stability exhibited by mammalian exosomes.[61] Likewise, other studies have shown that the prepared PDNVs can be stored at −80°C and used in subsequent experiments.[42,62] Notably, in a specific study, the authors tested the stability of Dendropanax morbifera leaf-derived PDNVs alone or with preservatives under various temperature conditions. Results indicated that PDNVs using the preservative TMO and stored at 4 ° C showed the most excellent stability and were more resistant to freeze-thaw cycles. Therefore, it is well worth considering adding preservatives to the solvents of PDNVs to improve their stability.[63]

Role of the HMDNVs in Cancer Therapy

In view of the excellent therapeutic advantages shown by PDNVs, more and more studies have been devoted to exploring the potential of PDNVS in cancer treatment and providing a basis for the development of new drugs for cancer treatment, especially those derived from plants with certain therapeutic activities (Table 1). The antitumor mechanisms of the following various PDNVs are shown in Figure 5.

Ginseng

Known as the "king of herbs", the use of ginseng in traditional Chinese medicine can be traced back more than 5000 years to Huangdi Neijing. Ginsenoside, the primary active component of ginseng, has been demonstrated to have a number of advantageous benefits, including anti-inflammatory, antioxidant, and anti-cancer effects. The results of clinical studies have shown that ginseng can improve psychological function, immune function, and diabetes-related conditions.[72,73]

Cao et al isolated GDNPs from ginseng root by sucrose gradient ultracentrifugation with a hydrodynamic size of about 344.8 nm and a negative zeta potential (− 25.4 mV), which contain various proteins, amino acids, lipids, nucleotides, and ginsenosides Rg3, the active component of ginseng. Moreover, they have shown that ginseng derived PDNVs induce apoptosis in murine melanoma cells by inducing polarization of M1-like macrophages through TLR-4/ MyD88 signaling and enhancing total ROS production.[42] In addition, their another further study show that ginseng derived PDNVs increase the release of CCL5 and CXCL9 from tumor-associated macrophages (TAMs) facilitates the recruitment of CD8+ T cells to the tumor site, which has a synergistic effect on PD-1 monoclonal antibody therapy without systemic toxicity detected.[64] These two studies provides a basis for ginseng derived PDNVs to be further used as nanomedicine for tumor immunotherapy.

Tea Flower

Tea plant, native to the Yunnan-Guizhou Plateau in southwest China, has a cultivation history of more than 5000 years. In traditional Chinese medicine, its flowers—known as tea flowers—are used for deodorization, skin care, cough alleviation, and phlegm. Tea flowers have a chemical make-up that is comparable to that of tea; they are mostly made of catechins, polysaccharides, proteins, amino acids, and saponins. As a result, it provides a range of health advantages, including antioxidant, anti-inflammatory, anti-tumor, hypoglycemic, and hypolipidemic properties as well as anti-allergic activity.[74,75]

Chen et al obtained teaflower derived PDNVs by sucrose density gradient centrifuge, derived from bands between 15% and 30% sucrose gradients. These PDNVs has a hydrodynamic size of 131 nm, a negative zeta potential (− 7.6mV), and a high concentration of polyphenols, flavonoids, functional proteins, and lipids. In terms of anticancer activity, teaflower derived PDNVs induced apoptosis of breast cancer cells mainly by inducing oxidative stress, leading to mitochondrial damage and cell cycle arrest in vivo. In vitro, teaflower derived PDNVs preferentially accumulate in breast tumor tissues and their lung metastasis sites, and inhibit breast tumor growth and lung metastasis by producing reactive oxygen species and regulating microbiota.[60]

Asparagus Cochinchinensis

Asparagus cochinchinensis (Lour). Merr. (A. cochinchinensis), as part of traditional Chinese medicine, A. cochinchinensis first appeared in Shennong's Classic of Materia Medica), the medicinal value of this plant has been proven by clinical studies over a long period of time. A. cochinchinensis' dried roots have long been used for treating asthma, cough, constipation, and thrombosis, either alone or in combination with other herbal medicines. To far, more than 90 chemicals from A. cochinchinensis have been isolated and described, with therapeutic actions ranging from anti-asthma to anti-inflammatory, antioxidant, anti-tumor, antidepressant, neuroprotective, and ameliorating Alzheimer's disease and intestinal illnesses.[76]

Zhang et al successfully extracted A. cochinchinensis-derived PDNVs by sucrose density gradient ultracentrifuge, which were mainly concentrated in bands between 30% and 45%, with an average size of 119nm, negative zeta potential (− 21mV), containing a variety of lipids, proteins and small RNAs, and showing typical cup-like structure. In addition,

Table 1 Characterization, Target Cancer and Mechanism of Action of Different CHMDNVs

| Plant	Size	Zeta Potential	Contents	Target Cancer (Cell)	Mechanism	Ref.
Ginseng	344.8 nm	−25.4 mV	Proteins, amino acids, lipids, nucleotides, and ginsenosides Rg3	melanoma	Increased apoptosis by promoting M2 to M1 phenotypic polarization and production of total reactive oxygen species.	[42]
Ginseng	200.4 nm	Unknown	Unknown	Breast cancer, Colon cancer	Increase the release of CCL5 and CXCL9 from TAMs facilitates the recruitment of CD8+ T cells to the tumor site.	[64]
Tea flower	131 nm	−7.6 mV	Polyphenols, flavonoids, functional proteins, and lipids	Breast cancer	Induce oxidative stress, mitochondrial damage and cell cycle arrest in vivo; produce reactive oxygen species and regulate microbiota in vitro.	[60]
Asparagus cochinchinensis	119 nm	−21 mV	Lipids, proteins and small RNAs	Liver cancer	Inhibit tumor growth, induce apoptosis, and up-regulate apoptosis-related factors.	[65]
Bitter melon	100~150nm	Unknown	Unknown	Oral squamous cell carcinoma	Induce cell apoptosis and enhance the therapeutic effect of 5-fluorouracil as well as reduce the resistance of OSCC to 5-fluorouracil.	[66]
Momordica charantia	120 nm	Unknown	miRNAs	Glioma	Inhibit glioma cell proliferation, migration and invasion in vitro by PI3K/AKT signaling pathway; Cross BBB and accumulate in tumor tissue to inhibit glioma growth in vivo.	[67]
Garlic	50~150nm	Unknown	Unknown	Kidney carcinoma cell, lung carcinoma cell	Increase the gene expression level of pro-apoptotic genes and decrease the anti-apoptotic genes; Block the cell cycle in S phase.	[68]
Fingerroot	100.2±10.1 nm	−26.9±6.1mV	Proteins, alkaloids, phenols, lipids, and organic compounds	Colorectal cancer	Perturb the equilibrium of intracellular redox processes and promoting programmed cell death	[69]
Dendropanax Morbiferus	100~200 nm	−25 mV	Unknown	Cancer-associated fibroblasts	Inhibit the growth of tumor-associated fibroblasts; Regulates the expression levels of genes related to growth factors and extracellular matrix components.	[70]
Moringa oleifera	240~500 nm	Unknown	RNAs, proteins, and lipids	Hela (cervical cancer cell), Jurkat (T lymphoblastic leukemia cells)	Downregulate the expression of Bcl-2 and induced a decrease in mitochondrial membrane potential.	[71]
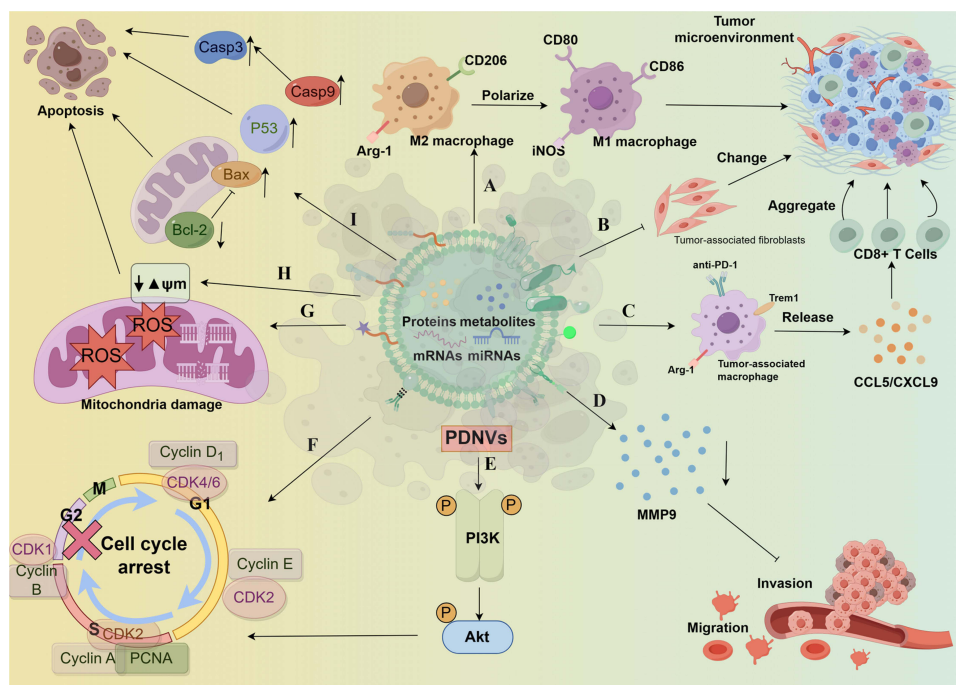
Figure 5 Mechanism of action of PDNVs against tumor cells. a. Promote M2 macrophage polarization are M1 macrophages, changing tumor microenvironment. b. Restrain the growth of the cancer associated fibroblasts, changing tumor microenvironment. c. Promote tumor associated macrophages release CCL5 / CXCL9 in order to promote gathered CD8 + T cells to the tumor. d. Reduce the release of MMP9 in inhibition of cell invasion and migration. e. Activated PI3K/Akt signaling pathway in inhibition of cell proliferation, invasion and migration. f. To block the cell cycle to inhibit cell proliferation. g. Promote the generation of mitochondrial ROS induce mitochondrial damage. h. Induction of mitochondrial membrane potential to reduce. i. Promote the expression of apoptosis factors, reduce the expression of apoptosis inhibiting factor. The figure was drawn by Figdraw.

the antitumor properties of A. cochinchinensis-derived PDNVs in vitro have been demonstrated in hepatocellular studies, demonstrating their ability to inhibit tumor growth, induce apoptosis, and up-regulate apoptosis-related factors.[65]

To conclude, as a promising alternative to A. cochinchinensis extracts, A. cochinchinensis-derived PDNVs may achieve satisfactory therapeutic benefits and better drug-like properties over their traditional counterparts.

Bitter Melon

Bitter melon is a vegetable commonly eaten in Asia, especially in India and China, and is distinguished by its bitter taste. In the meanwhile, bitter melon is used as a folk remedy to treat conditions including diabetes, eczema, pneumonia, as well as being an antiviral, anti-malaria, and anti-bacterial agent. In addition, more than 30 medicinal compounds are found in bitter melon, including proteins, peptides, and small compounds, some of which have anti-tumor, anti-diabetic, and anti-HIV properties.[77,78]

By electrophoresis and dialysis, Yang et al isolated bitter melon derived PDNVs from bitter melon juice, which could be taken up by oral squamous cell carcinoma (OSCC) cells and induce cell apoptosis. Strong anticancer activity was seen, with the impact being 100 times greater than that of bitter melon juice with a similar quantity of proteins and RNAs. Also, it has been demonstrated that bitter melon derived PDNVs can lessen OSCC resistance to 5-fluorouracil and improve the therapeutic impact of the drug.[66]

In addition, Wang et al also isolated the bands between 30% and 45% sucrose gradients by sucrose density gradient ultracentrifugation and obtained *Momordica charantia*-derived PDNVs with a size of about 120 nm and rich in multiple miRNAs. Furthermore, they found that these PDNVs inhibited glioma cell proliferation by arresting cell cycle through PI3K/AKT signaling axis, and inhibited U251 glioma cell migration and invasion by accelerating the epithelial-mesenchymal transition and decreasing MMP9 in vitro. Importantly, *Momordica charantia*-derived PDNVs cross the blood-brain barrier (BBB) and are enriched in tumor tissues, where they inhibit glioma growth and metastasis in vivo.[67]

Garlic

Garlic (*Allium sativum* L.) belonging to the family Liliaceae, is one of the oldest cultivated plants in the world, has been used for centuries for food and medicine. The medicinal use of garlic has been documented in ancient medical works of multiple civilizations, and in ancient China, garlic was one of the most commonly used Chinese herbal medicine since 2700 BC and was effective in treating most human diseases such as infections, cancer and heart disease.[79] According to modern medical research, garlic contains a number of bioactive compounds, such as organic sulfides, saponins, phenolic compounds, and polysaccharides, which have anti-inflammatory, anti-bacterial, anti-viral, anti-fungal, and anti-tumor properties.[80,81]

İrem Ozkan et al isolated small garlic-derived PDNVs between 50 and 150nm in diameter by using aqueous two-phase systems. Moreover, they found that garlic-derived PDNVs promoted cancer cell apoptosis by increasing the gene expression level of pro-apoptotic genes such as p53, Bax, Cas3 and Cas9 and decreasing the gene expression level of anti-apoptotic gene Bcl-2, while blocking the cell cycle in S phase to inhibit cancer cell proliferation, yet having little or no toxicity to normal cells.[68]

Fingerroot

Fingerroot, belonging to the Boesenbergia genera within the Zingiberaceae family, is a herb extensively distributed in Asian countries, where it is frequently employed as a culinary component and in ethnomedicinal formulations. Furthermore, investigations have revealed that fingerroot extracts and isolated compounds exhibit diverse pharmacological properties, including antiviral, anti-inflammatory, and anticancer activities.[82]

In a study, fingerroot-derived PDNVs were obtained from finger root juice through the implementation of differential centrifugation and size-exclusion techniques. Out of more than 30 fractions, fraction 8 exhibited the highest vesicle count, the most negative zeta potential value (-26.9 ± 6.1mV), and an average particle size of 100.2 ± 10.1 nm. Furthermore, fingerroot-derived PDNVs were found to contain various biomolecules such as proteins, alkaloids, phenols, lipids, and organic compounds. Notably, the presence of naringenin chalcone, pinostrobin, and pinocembrin in these PDNVs was identified, which was the bioactive constituents in finger root with anticancer activity. In addition, the findings of this study demonstrate that fingerroot-derived PDNVs possess a discerning ability to combat colorectal cancer cells by perturbing the equilibrium of intracellular redox processes and promoting programmed cell death. These results imply that fingerroot-derived PDNVs hold promise as an innovative therapeutic approach for individuals diagnosed with colorectal cancer.[69]

Dendropanax Morbiferus

The utilization of *Dendropanax Morbiferus*, a flowering plant belonging to the Araceae family and native to southern Asia, as a traditional remedy for diverse ailments has been well-documented. The therapeutic potential of extracts derived from the roots, leaves, and stems of *Dendropanax Morbiferus* encompasses the treatment of inflammatory conditions, diarrhea, diabetes, cancer, and certain microbial infections.[83]

To enhance the intracellular delivery efficiency and clinical effectiveness of bioactive compounds present in *Dendropanax Morbiferus* sap, a series of techniques involving continuous centrifugation and filtration, based on mammalian exosome separation technology, were employed for the isolation and acquisition of *Dendropanax Morbiferus* sap derived PDNVs with particle sizes between 100 and 200 nm and surface potentials of −25 mV. Additionally, it was observed that PDNVs derived from *Dendropanax morbifera* sap exhibited a dose-dependent inhibitory impact on cancer-associated fibroblasts (CAFs), pivotal contributors to cancer metastasis. Furthermore, these PDNVs regulated the expression levels of diverse genes, particularly those associated with growth factors and extracellular matrix components such as integrins and collagen. These results imply that PDNVs derived from *Dendropanax morbifera* sap hold promise as anti-CAF agents for diminishing CAF populations within the tumor microenvironment.[70]

In addition, their another study determined that the combined administration of *Dendropanax morbifera*-derived PDNVs and *Pinus densiflora*-derived PDNVs yielded superior efficacy compared to the sole use of *Dendropanax morbifera*-derived PDNVs, while also resulting in a reduced incidence of adverse reactions. This combination therapy involving *Dendropanax morbifera*-derived PDNVs and *Pinus densiflora*-derived PDNVs holds promise as a potential pharmacological regimen for tumor treatment, and future research endeavors should prioritize investigating the synergistic effects of *Dendropanax morbifera*-derived PDNVs in conjunction with other PDNVs.[84]

Moringa Oleifera

Moringa oleifera (MO), commonly referred to as the "tree of life" or "miracle tree", exhibits widespread growth in nearly all tropical and subtropical areas and is recognized as a significant botanical specimen owing to its extensive therapeutic and non-therapeutic advantages. Historically, this plant has been employed for the treatment of various ailments such as wounds, pain, ulcers, liver and heart diseases, cancer, and inflammation. To date, over one hundred compounds have been identified in MO, demonstrating robust antioxidant, anti-cancer, antihypertensive, hepatoprotective, and nutritional properties.[85]

Marina Potesta et al utilized MO seed powder as a primary material to produce MO aqueous extract (MOES). The acquired MOES underwent filtration using 0.45 μm and 0.22 μm pore size filters, and the resulting PDNVs were extracted through centrifugation at 13,000 × g for 5 minutes. The majority of the obtained PDNVs exhibited sizes ranging from 240 to 500 nm and contained RNA, protein, and lipids, but showed DNA deletion, indicating a comparable morphology and density to exosomes found in mammalian organisms. In tumor cell lines, treatment with MOES derived PDNVs resulted in a reduction in cell viability and an increase in apoptosis levels, concomitant with a downregulation of B-cell lymphoma 2 protein expression and a decrease in mitochondrial membrane potential. Notably, the effects observed with PDNVs treatment were comparable to those observed with MOES treatment and transfection using a pool of small RNAs isolated from MOES as a control.[71]

In conclusion, the utilization of MOES derived PDNVs, as an extract of MO, not only exhibits superior efficacy but also demonstrates significantly attenuated adverse effects compared to MO. This conclusion may offer a promising direction for further research and utilization of herbal medicine.

Clinical Trials of HMDNVs or PDNVs Loaded with Natural Active Products of CHM

In view of the excellent performance of HMDNVs as novel therapeutic agents and the promising anticancer effects shown in various studies, putting the potential HMDNVs into clinical use as early as possible is highly desired. However, as the research is still in its infancy, there are very few clinical trials on plant exosomes in progress or completed,[86] one of which aimed to see whether ginger or aloe derived exosomes could treat and improve the condition of Polycystic Ovary Syndrome, but was unfortunately withdrawn because no patients were enrolled. In another study, grape exosomes were investigated as an anti-inflammatory agent to reduce oral mucositis during radiotherapy and chemotherapy for head and neck cancer. The remaining two clinical trials related to HMDNVs are shown in Table 2.

Table 2 Two Clinical Trials Related to CHMDNVs

Trial Title	Condition or Disease	Intervention Model	Primary Outcome Measures	Start and Completion Date	NCT Number
Study Investigating the Ability of Plant Exosomes to Deliver Curcumin to Normal and Colon Cancer Tissue	Colon Cancer	Three Arms: 1. Curcumin; 2. Curcumin conjugated with plant exosomes 3. No treatment	Curcumin concentration in normal and cancerous tissue	January 2011 to January 2022	NCT01294072
Plant Exosomes ± Curcumin to Abrogate Symptoms of Inflammatory Bowel Disease	Irritable Bowel Disease	Three Arms: 1. Ginger exosomes; 2. Curcumin; 3. Ginger exosomes plus curcumin	Colonoscopy for inflammatory changes	March 1, 2018 to August 2, 2022	NCT04879810

Conclusions and Perspectives

Although the research on PDNV is still in its infancy, it has attracted wide attention as a new generation of therapeutic agents due to its widespread availability, ease of access, allowing large-scale production, and demonstrating its safety and stability in vivo and in vitro, as well as high cellular internalization rate. However, it is precisely because it is in the preliminary stage, it has to be admitted that there are still some problems in the present research of PDNVs.

Most of the current isolation methods first use a juicer to destroy plant tissues to obtain PDNVs. As a result, both EVs and artificial nanoparticles/microsomes formed by the fusion of ruptured membranes, as well as natural intracellular vesicles are isolated inevitably.[87] In addition, ultracentrifugation is a common method for exosome purification. Nevertheless, repeated precipitation of exosomes under high centrifugal force may damage membrane integrity or promote aggregation.[88] What's more, high centrifugal force also results in the formation of non-vesicular contaminants such as protein aggregates, lipoproteins and RNA bound to ribonucleoprotein complexes.[89] These problems may lead to errors in the content as well as functional analysis of PDNVs. Although the decreased centrifugal force is efficient in avoiding aggregation between vesicles and eliminating impurities, it will surely reduce the yield somewhat. Therefore, designing a better centrifugal force to achieve a satisfactory balance between yield and purity is worthy of further exploration. Moreover, an innovative study used plant tissue and cell culture techniques to obtain pure and authentic ginseng derived EVs,[90] which avoided tissue fragmentation and thus the generation of artificial nanoparticles/microsomes and intracellular vesicles. Of fact, this strategy also gives up the original simplicity and cost advantages of traditional methods, and additional study is needed to establish the practicality of this method.

According to the guidelines from Minimal information for studies of extracellular vesicles 2018 (MISEV2018).[91] Western blotting of exosomes' marker proteins is an essential step in identifying mammalian exosomes. Based on the origin and biogenesis of exosomes, membrane transport and fusion proteins, tetraspanin proteins, Heat shock proteins and vesicle-forming proteins are taken as its marker proteins, such as GTPases, CD9, CD81, HSP90, and TSG101.[92] For plant EVs, including PDNVs, there are not any mature or trustworthy marker proteins, though. In spite of Marcela Pinedo et al identifying three common protein families in plant EVs and considering three potential candidate marker proteins in 2021,[87] it was not universally regarded as accurate. Therefore, more plant EV proteomic analyses are still required to identify a set of plant EV protein markers that are conserved among plants, reliable, and widely recognized. Moreover, there are no commercially available antibodies against these candidate markers at present.

Another prominent issue is the confusion regarding the nomenclature of PDNVs, which, as was mentioned before, varies throughout the literature. For example, turmeric derived exosome-like nanovesicles, ginger derived nanoparticles, A. cochinchinensis derived nanovesicles, bitter melon derived extracellular vesicles, garlic-derived SEVs, etc. In this review, in order to highlight their similarities with mammalian exosomes in morphological structure (phospholipid bilayer nanovesicles), contents (lipids, proteins, RNA), and function (intercellular communication, immune defense), we have named them plant-derived exosome-like nanovesicles.

It is worth mentioning that given their excellent stability, safety, biocompatibility, and ability to facilitate large-scale manufacture, PDNVs are also exploited as drug delivery vectors. For maximum economic benefit, these vectors are usually made from relatively inexpensive plants, such as grapefruit, ginger, cabbage,[93] and lemon.[94] Among them, grapefruit and ginger derived PDNVs are the most widely studied delivery vectors. Due to its outstanding delivery ability, the former has been studied for the delivery of doxorubicin plus heparin,[95] curcumin plus doxorubicin,[96] miRNA[97] and siRNA plus paclitaxel as well as JSI-124 (Cucurbitacin I)[98] to treat glioma, colon cancer, breast cancer and other diseases. Ginger derived PDNVs have been investigated for the delivery of doxorubicin[99] and siRNA[53,100] to treat colon cancer due to their delivery ability and own therapeutic properties, which often produce synergistic effects to enhance the therapeutic effect. In addition, PDNVs have been modified to enhance their targeting ability or loading capacity, which is equally an attractive aspect of PDNVs and a popular area for investigation.

Since HMDNV frequently inherits certain features of the original herbal medicine, we recommend that future research on HMDNV begin with a focus on the previous pharmacological effects of the selected herbal medicine and the therapeutic activities of the natural active products contained in it, and then go on to the corresponding illness type for investigation, or vice versa. This is not an inherently uninnovative model; rather, it is a vital step toward developing

novel uses of herbal medicine and their natural active components in the treatment of illnesses and malignancies, as well as an efficient strategy to address the bottleneck in their current research. Of course, it would be preferable to discover new therapeutic activities of HMDNVs in a novel manner. What's more, there is growing evidence that the therapeutic efficacy of HMDNV is frequently superior to that of the same dose of the natural active substance itself and the herbal juice containing the same dose. For example, cucumber sarcocarp-derived PDNVs containing the same dose of CuB exhibited more potent anticancer activity than free CuB;[50] In comparison with bitter melon juice containing the same concentration of proteins and RNAs, bitter melon derived PDNVs showed 100 times greater anticancer activity.[66] This might be because of the vesicular structure, which improves the stability and bioavailability of the active compounds, or because the complex and varied components in PDNVs synergistically boost anticancer action. This also raises a new issue: the components in PDNVs are unknown and complicated, potentially posing safety issues. As a result, even though the majority of PDNVs are generated from edible plants, each member of the PDNVs family should undergo safety testing.

Herbal medicine has been enduring for thousands of years, with its unique effect on some difficult and complicated diseases. Nonetheless, there is still a great deal of room for the development of HMDNVs and its use in the treatment of cancer as the present study on HMDNVs is relatively scant. Only in China, it is reported that there are more than 10,000 prescriptions of Chinese herbal medicine recorded in various ancient Chinese medical works,[101] and according to the previous national natural resources survey, there are a total of 12807 kinds of Chinese herbal medicine,[102] which will be a huge treasure trove full of surprises for the development of new anticancer nanomedicine.

Abbreviations

PDNVs, Plant-derived exosome-like nanovesicles; HMDNVs, herbal medicine derived exosome-like nanovesicles; EPR, enhanced permeability and retention effect; EVs, extracellular vesicles; MVBs, Multivesicular bodies; TEM, transmission electron microscopy; LEs, late endosomes; ILVs, intraluminal vesicles; ESCRT, endosomal sorting complexes required for transport; TOL, TOM1-like; PA, phosphatidic acid; PC, phosphatidylcholines; PE, phosphatidylethanolamine; DGDG, digalactosyldiacylglycerol; MGDG, monogalactosyldiacylglycerol; PI, phosphatidylinositol; BASP1, Brain Acid-Soluble Protein 1; IDO1, indoleamine 2.3-dioxygenase 1; Cer, ceramide; ROS, reactive oxygen species; CD98, cluster of differentiation 98; miRNA, microribonucleic acid; 3'-UTRs, 3'-untranslated regions; FDA, food and drug administration; TAMs, tumor-associated macrophages; OSCC, oral squamous cell carcinoma; BBB, blood-brain barrier; CAFs, cancer-associated fibroblasts; MO, Moringa oleifera; MISEV2018, Minimal information for studies of extracellular vesicles 2018.

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Disclosure

The authors report no conflicts of interest in this work.

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