GUHES 2025; 7 (1): 19-27
DOI: 10.59124/guhes.1600806

Review

Journal of Gazi University Health Sciences Institute

journal homepage*:* https://dergipark.org.tr/tr/pub/guhes

Plant-Derived Exosome-Like Nanoparticles Based Treatments In Cancer Therapy

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Article info:

Received: 26.12.2024

Accepted: 01.03.2025

Keywords:

exosome,
cancer,
characterization,
ısolation,
PDELNs,

Abstract

Plant-derived exosome-like vesicles (PELVs) are nanometer-sized particles comprising proteins, lipids, nucleic acids, and small molecule substances generated from plants. PELVs have many advantages, such as low toxicity, efficient cellular uptake, high biocompatibility, stability, and large-scale production. PELVs can regulate intercellular communication by releasing their contents, including mRNA, miRNA, lipids, and proteins. Plant-derived exosome-like vesicles (PDELVs) have attracted considerable attention in scientific research owing to their promising therapeutic effects and researches have assessed the the extensive therapeutic potential of PDELVs in the treatment of various diseases including cancer treatment. They exhibit various clinical attributes and therapeutic benefits over conventional pharmaceuticals. This mini-review aims to summarize and categorize the main paths followed by scientists working with the PDELNs for cancer therapy.

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Citation: Umurhan, G., Esmekaya M.A., Ertekin B., & Tomruk A. (2025). Plant-derived exosome-like nanoparticles based treatments in cancer therapy. *Journal of Gazi University Health Sciences Institute*, 7(1), 19-27. https://doi.org/10.59124/guhes.1600806

1. Introduction

Scientists have focused on nano-sized biological materials that are originated from human body to achieve a successful and effective treatment in the targeted cancer therapy. The studies conducted on cancer treatments showed that various structural and functional factors of these nano-sized materials affect the molecular systems: their synthesis, releases, sizes, and contents. It might be interesting that recent studies revealed that there are compositional similarities between plant and mammalian derivatives nano-sized vesicles. For this reason, scientists have turned to using the plant derivative tiny lipid bilayers for their medical treatments and therapeutics. Because of their high biocompability and non-toxicity to human, these plant derived exosome-like nanoparticles (PDELNs) can easily trigger the immune response and inflammation. These nanoparticles can participate in many physiological process to regenerate and control the human cancerous or inflammatory tissues (Zhang et al., 2021). Therefore, scientists have recently focused on PDELNs to identify and improve the usage area of these PDELNs vesicles in cancer therapy.

2. Content of PDELNs

Mammalian cell-derived exosomes have a lipid bilayer that is mostly composed of phosphotidylserine, ceramides, cholesterol, and glycosphingolipids, which give them stability and a special rigidity. On the other hand, PDELN membranes are abundant in digalactosyldiacylglycerol (DGDG), phosphatidic acid (PA), phosphatidylcholines (PC), and monogalactosyldiacylglycerol. These unique lipid characteristics offer inherent regulatory functions for mammalian cells. A study found that ginger-derived exosomes include 25-40% of total lipids as phosphatidic acid, 25-40% as galactosyldiacylglycerol, and 20-30% as monogalactosyldiacylglycerol. (Zhang et al., 2016). One phospholipid that distinguishes out among the others is phosphatidic acid, which is frequently present in PDELNs and helps target and stimulate the mTOR pathway, which is in charge of cell growth, proliferation, and repair. By establishing a cellular block in the cell membrane, phosphatidylcholines, a source of choline in the body, can shield a colon cell wall (Kim et al., 2022). The morphology and lipid composition of PDELNs are critical for intestinal cell absorption. PDELNs have large amounts of phospholipids, whereas mammalian exosomes are rich in cholesterol and sphingomyelin. (Suharta et al., 2021).

The regulation of glycolipid metabolism, membrane-associated proteins, and cytosolic proteins is controlled by the structural proteins of PDELNs vesicles. According to the literature, large number of confirmed protein types have been detected in PDELNs, such as heat shock proteins considered exosome markers (e.g. CD9 and CD63), transmembrane proteins (e.g. actin, proteolysis, aquaporin, and chloride channel proteins), defense proteins, and other plasmalemma-associated proteins. BKEBN obtained from bitter orange, grapefruit, lemon, and sweet orange was studied and shown to have a considerable amount of proteins (Sarasati et al., 2023, Sha et al., 2024).

Other significant discovery for exosomes is the microRNA (miRNA, 19–24 nt, non-coding RNAs) used for targeted drug delivery systems. MicroRNAs regulate the expression of numerous mRNAs, which is critical in many biological processes. They may also impact the course of certain illnesses by facilitating cell-to-cell communication. miRNAs are involved in all cellular processes and are critical for differentiation of cells and homeostasis. They are released into the extracellular fluid and can serve as biomarkers for the identification of various illnesses. (Asgarpour et al., 2020). miRNAs identified in PDELNs can control gene expression via guiding mRNA cleavage. A research study found that miR168a generated from rice can precisely target and regulate LDLRAP1 (low-density lipoprotein receptor adaptor protein 1) expression in mice livers. In another study, it was demonstrated that plant miR159 inhibits the growth of breast cancer cells by targeting the TCF7 gene. It is possible that the miRNA carried by PDELNs determines their functions. Additionally, miRNA patterns in PDELNs vary by plant species, implying that ELNs derived from diverse edible plant sources may serve different regulatory roles (Leng et al., 2024).

3. Isolation of PDELNs

Numerous methods for the isolation of exosomes in a significant quantity and purity have been developed thanks to the rapid advancements in science and technology. In order to facilitate exosome isolation, each method takes advantage of a specific characteristic of the particles, like shape, density, size, and surface proteins (Lie et al., 2017). Techniques for isolating exosomes, which are essential in biological research, have advanced significantly. Exosome isolation techniques are generally categorized as follows.

3.1. Ultracentrifugation

This method has established the gold standard for isolating and purifying ELNs, thanks to its simplicity and low cost. The technique relies on the variations in the size and density of the separated particles. Centrifugation is commonly used for the separation and purification of particulate materials, as well as to investigate the hydrodynamic properties of polymeric materials, comprising biopolymers like DNA and proteins. A suspension's particles are successively separated based on their physical characteristics as well as the solvent's density and viscosity, all of which are influenced by the centrifugal force that is applied.

Ultracentrifugation is expected to account for 80% of all exosome isolation techniques utilized in exosome research. Many people utilize ultracentrifugation as an isolation method because it is simple to use, requires little technical experience, is cost-effective over time (e.g., an ultracentrifuge for long-term usage), and requires little or no sample pretreatment. Because of these factors, researchers studying exosomes are increasingly choosing to use ultracentrifugation-based methods. (Li et al., 2017, Omrani et al., 2024, Miron et al., 2024).

3.2. Differential Ultracentrifuge

Differential ultracentrifugation is another centrifugation-based method. In summary, the exosome source, biological fluid or plant broth, is centrifuged at escalating speeds and durations to

remove larger and higher-density components; the resulting pellets are discarded. The supernatant is centrifuged at 100,000 × g or more to recover the exosome-containing pellet, which is subsequently resuspended and washed in a buffer. This method is frequently adapted for different exosome sources. (Akuma et al., 2019).

3.3. Density Gradient Centrifugation

Density gradient ultracentrifugation is now widely used to isolate extracellular vesicles such as exosomes. Exosomes are separated via density gradient ultracentrifugation based on their size, mass, and density in a centrifuge tube's pre-configured density gradient medium, where the density progressively drops from bottom to top. After a lengthy ultracentrifugation cycle, a sample is layered as a narrow band on top of the density gradient medium. Following the application of centrifugal force, the sample's solutes—including exosomes—migrate as distinct zones across the density gradient medium, each moving toward the bottom at its own rate of sedimentation. This results in the formation of distinct solute zones. The divided exosomes can then be simply retrieved using simple fraction. One of the drawbacks of density gradient ultracentrifugation, in contrast to differential ultracentrifugation, is that its capacity is mostly restricted to the narrow load zone (Zhang et al. 2018).

3.4. Size Exclusion Chromatography (SEC)

The method employs a porous gel filtration polymer as the stationary phase and the original biofluid as the mobile phase. Differential elution is possible due to the stationary phase's structure. Large particles are extracted first, then smaller vesicles, and finally proteins not bound to the membrane. Because larger particles can only pass through fewer pores, they travel shorter distances to the end of the column and are separated faster than their smaller counterparts. SEC separates small and big vesicles and removes pollution from soluble proteins that are not attached to exosomes. As a result, the exosome is pure, undamaged, and reasonably priced. Nevertheless, exosomes and identically sized microvesicles cannot be distinguished by SEC. (Sidhom et al., 2020).

3.5. Ultrafiltraiton

The current generation of commercial membrane filters makes it easier to isolate particles of a specific size due to their small pore size distribution and range of sizes. Micro- or ultrafiltration is frequently employed in conjunction with an ELN isolation approach by researchers. Ultrafiltration, in instance, can be utilized as a step between successive ultracentrifugation stages and gel filtration chromatography. However, only microfiltration and ultrafiltration are suitable for ELN isolation. (Konoshenko et al., 2018).

3.5.1. Precipitation

Following ultracentrifugation, the approach based on ELN precipitation in PEG (Polyethylene glycol) solutions appears to be the most popular. This technique exploits chemicals' decreased solubility in PEG solutions, which are superhydrophilic polymers. The technique involves combining the sample with the polymer solution, incubating it, then precipitating the ELNs using low-speed centrifugation (1500 ×g). The

PEG approach allows for the simultaneous processing of huge numbers of samples. The technique is simple, rapid, and scalable. (Konoshenko et al., 2021).

4. Characterization of PDELNS

After the purification of PDELNs vesicles, scientists must be characterized their biological materials depending on four categories: size, concentration, purity, and content.

4.1. Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM)

With the capacity to identify tiny exosomes, transmission electron microscopy (TEM) is the most efficient method for researching the morphology and structure of exosomes. The negative staining method is simple and quick, taking only a few hours, and the TEM resolution is around 1 nm.

Scanning electron microscopy (SEM) uses a fine-pointed beam rather than a broad beam like TEM to analyze samples line by line. As a result, SEM concentrates on the surface of the materials, producing a three-dimensional image of the exosomes rather than the two-dimensional image obtained by TEM. (Dilsiz et al., 2024).

4.2. Nanoparticle Tracking Analysis (NTA)

Biophysical approaches are utilized to determine the exosomal size range. NTA, an optical particle tracking device, is one such biophysical method that can quantify the concentration and size distribution of exosomes in the 10 nm to 2 μm range. The exosomal motion path is identified to determine the particle speed. This approach tracks the Brownian motion of nanoparticles in a liquid suspension at the particle level. NTA analyzes exosome mobility by tracking each particle using image analysis. The motion can then be connected to particle size. The size, concentration, size distribution, and phenotypic of the particles can all be ascertained from the findings of this technique (Alzhrani et al., 2021 ; Gurunathan et al., 2019).

4.3. Atomic Force Microscopy (AFM)

Another technology used in the ELN characterization procedure is AFM (Atomic Force Microscopy). It enables the high-resolution analysis needed to investigate EV structure while allowing for the in situ assessment of label-free samples with minimal sample preparation. By detecting the contact between a probe tip and the sample surface, AFM can determine the size distribution, concentration, and form of EVs. (Gazze et al., 2021).

4.4. Dynamic Light Scattering (DLS)

Dynamic light scattering (DLS) is an optical analysis technique used to determine the size and distribution of submicron particles. It is impossible to display details regarding the amount or concentration of a certain particle type because DLS analyzes each particle in a sample instantly. DLS indicates a defined range for EV diameter, however it is difficult to identify the concentration. As a result, to finish categorizing EVs, DLS technology is generally used in conjunction with other technologies. (Wu et al., 2024).

4.5. Flow Cytometry

Flow cytometry is a technique that captures the fluorescence and scattering signals generated by individual particles passing through a flow chamber under the illumination of a laser beam.. Because of its capacity to analyze multiple characteristics at the same time, flow cytometry is one of the most commonly used technology for studying exosomes. The device can count particles larger than 500 nm, which is useful for detecting microvesicles and apoptotic bodies. Because of side detection limits, typical cytometers may overlook nanoparticles less than 300 nm. (Rupert et al., 2017; Kurian et al., 2021).

4.6. Zeta Potential Measurement

The measurement of zeta potential (ZP), a measure of colloidal stability, is influenced by the electrophoretic mobility and surface charge in a suspension. Nanoparticles that are electrically charged are found in dispersed systems including emulsions, suspensions, and colloidal dispersions. ZP regulates the net surface charge of the nanoparticles in these dispersed systems, as well as the stability of the interactions between the particles and the medium, including the propensity of the particles to aggregate. As a result, ZP is one of the most valuable techniques for studying nanoparticle collective behavior, including colloidal stability, as shown in extracellular vesicles in dispersed systems. Similarly, ZP is one of the strategies used to evaluate the activity of extracellular vesicles in biological reactions. (Midekessa et al., 2020). Higher values of the Zeta potential indicate a more stable system of smaller dispersed particles, whereas lower absolute values indicate a stronger propensity to coagulate or concentrate. (Zhu and He, 2023).

Extracellular vesicles have a multicomponent structure containing small molecules such as proteins, lipids and nucleic acids and various high molecular weight materials. Recent studies have used spectroscopic techniques such as Raman (Guerrini et al., 2021), LC-MS (Zhao et al., 2024), GC-MS (Irıawati et al., 2024) and FTIR (Soares Martins et al., 2020) to evaluate the chemical composition of exosomes.

5. Biological Effects of PDELNs on Different Cancer Types

Stanly et al. investigated the anticancer effects of *ELNs isolated from grapefruit* on the A375 human melanoma cell line. The study reported that EBNPs caused a pause in the G1 phase of the A375 cell cycle and inhibited cell proliferation (Stanly et al., 2020). Kim et al. found that ELNs obtained from carrots helped neutralize free radicals in the cell and effectively reduced oxidative stress in cardiomyoblast and neuroblastoma cells by increasing the activity of antioxidant enzymes (Kim et al., 2021).

Studies have investigated the effects of PELNs on various types of cancer. In one study, *lemon-derived ELNs* were shown to arrest the S phase of the cell cycle and induce apoptosis in stomach cancer (Yang et al., 2021), while they were found to induce trace-mediated cell death in colon, blood, and liver cancer. They were also shown to mediate the inhibition of lipid metabolism and down-regulate Acetyl-CoA Carboxylase 1 in colon and blood cancer (Raimondo et al., 2018).

Kim et al. showed that *ELNs isolated from Panax ginseng* can effectively cross the blood-brain barrier, inhibit the growth and proliferation of glioma cells, and increase the apoptosis of tumor cells with both in vitro and in vivo studies (Kim et al., 2023). There are also studies in the literature on the effects of ginseng-derived ELNs on skin, colon, and breast cancer. ELNs were found to change macrophage polarization in skin cancer studies (Cao et al., 2019), and programmed cell death protein-1 monoclonal antibody and cold tumor environment were found to change in colon and breast cancer (Han et al., 2022).

The activity of *ELNs isolated from bitter melon* (BMELNs) in various cell lines has also been investigated. In one study, it was found that BMELNs inhibited the proliferation, migration, and invasion of glioblastoma cells by regulating the PI3K/AKT signaling pathway (Wang et al., 2022). Another study showed that BMELNs arrested the S-phase cell cycle of oral cancer, induced apoptosis, and reduced the resistance of cancer cells to 5-Fluorouracil by reducing NLRP3 expression (Yang et al., 2021).

In a study where *ELNs obtained from the leaves of the Gundelia munzuriensis* plant were applied to lung cancer (A549) and colon cancer (HCT116) cell lines, it was observed that they affected the viability of HCT 116 cancer cells by 40-50%, while a 50% decrease was observed in A549 cell lines (Demirhan et al., 2023). In another study aimed at investigating the cytotoxic effects of exosome-like nanoparticles (GdELN) obtained from the Gundelia Dersim plant native to Turkey on cell viability in human lung cancer (A549) and human colon cancer (HCT 116) cell lines, it was observed that isolated GdENs increased the concentrations of GdELNs that showed cytotoxic effect by reducing cell viability at different doses in HCT 116 cells (Erman et al., 2023).

Potesta et al., 2020 showed that *moringa oleifera-derived ELNs* induced apoptotic cell death in blood and cervical cancer cells. Ozkan et al. reported that garlic-derived ELNs caused caspase-mediated apoptosis in kidney and liver cancers (Ozkan et al., 2021). Another study found that corn-derived ELNs inhibited the proliferation of colon cancer cells and activated the release of tumor necrosis factor-α in macrophages (Sasaki et al., 2021).

It has been reported that *ELNs from Dendropanax morbifera* reduce cancer-associated fibroblasts around the tumor in skin cancer and prevent metastasis, while its use together with ELNs from Pinus densiflora has been reported to have anticancer effects in breast and skin cancer (Kim et al., 2020).

Liu et al. have shown that miR159 found in *soybean-derived ELNs* significantly reduces colon tumor formation in mice. These findings have shown that miR159 suppresses the expression of MYC oncogene in colon cancer cells in vitro using TCF-7-mediated signaling pathways (Liu et al., 2021).

It has been shown that hibiscus induces apoptosis in prostate cancer cells, especially in the LNCaP cell line. This effect has been observed in both in vitro and in vivo experiments. In animal experiments, specifically xenograft experiments in 8-week-old mice, mallow extract significantly inhibited tumor growth (Hui-Hsuan et al., 2012).

Ethical Statement

Ethics committee approval was deemed unnecessary for this study, given that open-access sources were utilized.

Financial Support for the Study

This study did not receive any financial support.

Presentation Information

The findings of this study have not been presented at any conference or journal.

Conflicts of Interest

The authors declare no conflicts of interest regarding this study. Any institution or organization providing funding for this research did not have any role in the design, data collection, analysis, interpretation, or publication to influence or distort the findings.

Author Contributions

The authors contribute as follows: Gaye Umurhan contributed to the literature search and editing of the report. Burhan Ertekin and Arın Tomruk revised the manuscript and Meriç Arda Eşmekaya conducted the supervision and final revision of the manuscript.

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