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# Comparison of nanovesicles derived from *Panax notoginseng* at different size: physical properties, composition, and bioactivity

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**Aim:** Plant-derived nanovesicles have emerged as potential agents for combating tumors. In this study, we investigated the inhibitory effects of *Panax notoginseng*-derived nanovesicles (PnNVs) on the proliferation and migration of squamous cell carcinoma. Additionally, we explored the relationship between plant tuber size and the physical properties, composition and bioactivity of these nanovesicles.

**Methods:** We isolated PnNVs from *Panax notoginseng* tubers of varying sizes: small-sized (s\_PnNVs), medium-sized (m\_PnNVs) and large-sized (L\_PnNVs), and evaluated for size, potential, and morphology. Cellular uptake efficiency was assessed using confocal microscopy and flow cytometry. The ability of different PnNVs to inhibit oral squamous cell carcinoma cells was evaluated using plate cloning, CCK8 assay, and scratch healing assay. Off-target metabolomics was used to compare metabolic compounds of different PnNVs.

**Results:** Our findings revealed that s\_PnNVs exhibited lower potential but had the highest cellular uptake efficiency, whereas m\_PnNVs were characterized by the smallest size and lowest cellular uptake efficiency. Notably, m\_PnNVs demonstrated the most effective inhibition of squamous cell carcinoma growth and migration. Compositional analyses showed that PnNVs were rich in proteins and contained lower levels of RNA, with L\_PnNVs having the highest protein content. Furthermore, untargeted metabolomics analysis revealed a significant increase in the expression of specific antitumour-related metabolites in m\_PnNVs compared to s\_PnNVs and L\_PnNVs.

**Conclusion:** Overall, our results underscore the influence of plant tuber size on the bioactivity of the nanovesicles from which they are derived, emphasizing its importance for experimental design and study reproducibility.

## KEYWORDS

plant derived nanovesicles, *Panax notoginseng*, squamous cell carcinoma, tuber size, composition, bioactivity

## Highlights

1. *Panax notoginseng* derived nanovesicles (PnNVs) inhibit squamous cell carcinoma proliferation and migration, with varying potency from different size.
2. PnNVs from *Panax notoginseng* at different size differ in morphology, potential distribution, particle size distribution, and composition.
3. PnNVs from *Panax notoginseng* at different size show different cellular endocytosis efficiency.

## 1 Introduction

Plant-derived nanovesicles (PDNVs) are lipid bilayer vesicles derived from plants, comprising plant-derived lipids, proteins, RNA, and metabolic compounds (Chen et al., 2023a). Serving as a natural drug encapsulation system, PDNVs possess the capability to improve drug bioavailability and exhibit enhanced cellular uptake owing to their lipid bilayer structure (Niu et al., 2021; Xiao et al., 2022). Furthermore, PDNVs are highly adaptable and can function as targeted drug delivery vehicles, showcasing considerable promise for utilization in fields such as antitumor treatment and tissue regeneration (Chen et al., 2023a; Chen et al., 2023b). Numerous studies have highlighted the potential of PDNVs in the realm of anti-tumor research (Ly et al., 2023), with examples including bitter melon (Yang et al., 2021), cucumber (Chen et al., 2022), and artemisia annua (Liu et al., 2023). The exploration for PDNVs exhibiting anti-tumor properties holds significant promise.

*Panax notoginseng*, a plant known for its diverse array of over 200 compounds with demonstrated antitumor (Zhang et al., 2022), anti-inflammatory (Zheng et al., 2022), haemostatic (Liu et al., 2014), and antioxidant properties (Xu et al., 2023a), is commonly utilized in herbal formulations like Yunnan Baiyao (Zhu et al., 2017). Of note, the tuber of *Panax notoginseng* plays a crucial role in contributing to its medicinal efficacy (Zheng et al., 2022). Previous studies have isolated nanovesicles from *Panax notoginseng* tuber (PnNVs) and demonstrated their ability to mitigate ischemia-reperfusion injury by modulating microglia polarization (Li et al., 2023). Furthermore, compounds from *Panax notoginseng* tuber, such as *Panax notoginseng* saponins and other components, have shown promising antitumor activity against hepatocellular carcinoma (Liu et al., 2021a), and breast cancer (Wang et al., 2014). Nevertheless, the anti-tumor efficacy of PnNVs, as potent herbal agents, remains incompletely understood. Squamous cell carcinoma is the predominant tumor pathology, prompting our initial assessment of the efficacy of PnNVs in suppressing the proliferative and migratory characteristics of squamous cell carcinoma. Our investigation revealed variations in the inhibitory effects of PnNVs based on their size, raising questions about the influence of factors such as tuber size on the bioactivity of these nanovesicles.

Numerous studies on PDNVs currently suffer from a lack of standardized criteria for plant selection, leading to potential challenges in study reproducibility. For example, certain plants such as turmeric (Gao et al., 2022) and ginseng

(Kim et al., 2023) are commonly utilized for their tubers in the isolation of nanovesicles, yet the impact of varying sizes on the physical properties and bioactivities of PDNVs is frequently overlooked. It is essential to consider factors such as growth stage, and other variables, as these may significantly influence the composition and concentration of active compounds within PDNVs (Liu et al., 2020; Han et al., 2023). Hence, it is imperative for plant selection to consider these factors in order to ensure the validity of research findings.

To fill this research void, *Panax notoginseng* was chosen as the focal point of investigation. This study presents the initial comprehensive examination of nanovesicles derived from *Panax notoginseng* tubers of varying sizes, encompassing distinctions in physical attributes such as shape, dimensions, and zeta potential dispersion, variances in cellular uptake and bioactivity, and distinctions in the content composition. The primary objective of this study was to provide a theoretical basis for the efficacy of PnNVs and the potential for identifying novel therapeutic uses for PnNVs. Additionally, emphasis was placed on the careful selection of tuberous plants in subsequent experimental design to enhance the replicability of the study in future research endeavors.

## 2 Materials and methods

### 2.1 Cell culture

The SCC-7 cells, a mouse squamous cell carcinoma cell line, were obtained from Yimo Bio (Xiamen, China), while the LN4 cells were derived from the CAL-27 cell line and purified following four rounds of lymph node metastases. Our research group previously engineered this cell line to exhibit heightened lymph node metastatic properties. The cell line was confirmed through short tandem repeat (STR) analysis (Gan et al., 2018). Both cell lines were maintained in Dulbecco's modified eagle's medium (DMEM) (Gibco) supplemented with 10% fetal bovine serum (FBS) (PAN) at 37°C in a 5% CO<sub>2</sub> incubator. Passages were conducted through trypsin digestion (Gibco) upon reaching 80% cell confluence.

### 2.2 Isolation of PnNVs

*Panax notoginseng* of varying sizes was obtained from a single farm in Wenshan, Yunnan, China. The fresh *panax notoginseng* tuber were initially washed with water and then rinsed with distilled water. Subsequently, the tubers were diced, squeezed, and filtered, to produce a supernatant. The supernatant underwent sequential centrifugation steps at 2,000 g for 10 min, 5,000 g for 20 min, and finally at 10,000 g for 30 min, with each step involving the separation of sediment and preservation of the supernatant. The supernatant was subjected to centrifugation at 100,000 g for 30 min to prevent nanovesicle aggregation (Chen et al., 2022), followed by resuspension in phosphate-buffered saline (PBS). This resuspended solution underwent two subsequent rounds of centrifugation at 100,000 g for 30 min. The resulting precipitate was resuspended in PBS solution, filtered through a 0.22 μm filter membrane, and stored at 4°C in a refrigerator.

## 2.3 Physical characterization of PnNVs

The nanovesicles were characterized morphologically using transmission electron microscopy (FEI TecnaiG2, Japan). The PnNVs were fixed with 4% paraformaldehyde by placing a copper mesh on top and inverting it for 20 min, followed by three washes with ddH<sub>2</sub>O. Subsequently, the samples were stained with a 2% phosphotungstic acid solution (pH = 7) for 2 min and washed three more times with ddH<sub>2</sub>O before being dried for further observation. Particle size and zeta potential distribution were determined using dynamic light scattering (DLS) (Anton Paar, Austria).

## 2.4 Cellular uptake of PnNVs

Cells were initially cultured in 12-well plates to allow for adherence. Subsequently, PnNVs, labeled with DIO fluorescent dye (UElandy), were added to the cells, and the uptake of PnNVs was observed via confocal microscopy (Olympus, Japan) after a 24-h incubation period. The cytoskeleton was stained with Phalloidin (UElandy), while the nucleus was stained with DAPI (Beyotime). Furthermore, cells were cultured in 6-well plates and exposed to DIO-labeled PnNVs. The efficacy of cellular uptake of PnNVs was assessed at 1, 12, and 24 h using flow cytometry (BD Accuri™ C6 plus, America).

## 2.5 Cell proliferation assay

Cells were cultured in 96-well plates, followed by replacement of the medium after 24 h. Subsequently, varying concentrations of PnNVs were added into the wells. A 10 μL CCK-8 working solution (Invitrogen) was then added, and after 1-h incubation period, optical density values were assessed at 450 nm to determine cell viability.

Cell viability was quantified using the formula:

$$\text{Cell viability (\%)} = \frac{(\text{optical density (OD)}_{\text{treated sample}} - \text{OD}_{\text{blank}})}{(\text{OD}_{\text{control sample}} - \text{OD}_{\text{blank}})} \times 100\%$$

## 2.6 Plate cloning experiment

Cells were initially plated in 6-well plates and incubated for 24 h before the medium was removed. Subsequently, the cells were treated with PnNVs at a concentration of 25 μg/mL. Upon the formation of clones, the cells were rinsed with PBS, fixed with 4% paraformaldehyde, stained with crystal violet (Beyotime) for 10 min, washed with running water, and finally imaged and recorded.

## 2.7 Scratch experiment

Cells were cultured in 12-well plates until reaching 95% confluence, at which point a cross-shaped scratch was created using a sterile P-200 pipette tip. Following PBS washing, PnNVs were added at concentrations of 25 μg/mL and 50 μg/mL. Images were captured before and after PnNVs addition, as well as during healing of the cell at the same position.

The wound healing rate was calculated using the formula:

$$\text{Wound healing rate} = \frac{(\text{initial blank area} - \text{healed blank area})}{(\text{initial blank area})} \times 100\%$$

## 2.8 Compositional identification of PnNVs

### 2.8.1 RNA

RNA was extracted from PnNVs by lysing the PnNVs with trizol (Invitrogen), followed by extraction, precipitation, and washing steps using chloroform, isopropanol, and 75% anhydrous ethanol (prepared with DEPC water), respectively. The RNA was then quantified using a Nanodrop (Thermo, United States). Subsequently, a 1% agarose gel was prepared, and 1 μg PnNVs' RNA was loaded onto the gel. Electrophoresis was carried out at 110 V for 30 min, and images were captured for analysis (Bioscience, China).

### 2.8.2 Proteins

Proteins were extracted from PnNVs through a process involving lysis with 5 × SDS lysate and sonication, resulting in homogeneous samples. These samples were then subjected to electrophoresis at 150 V for 60 min using a vertical electrophoresis device. The gels were stained with Coomassie brilliant blue and decolorized over a 24-h period. Photographs were taken and documented.

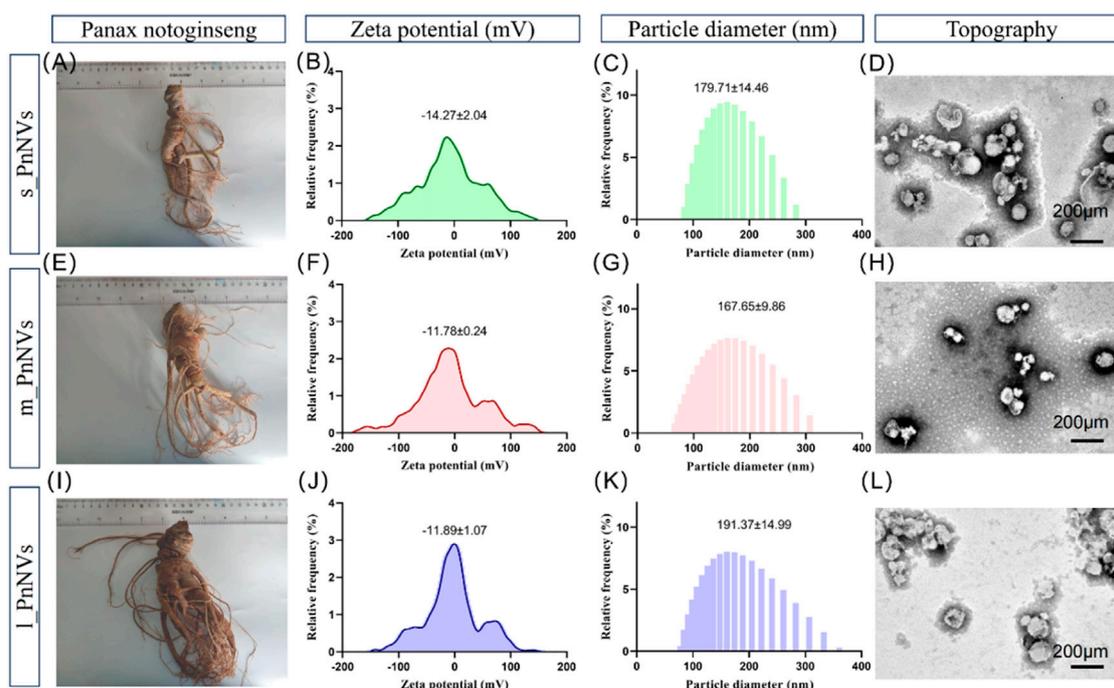
Furthermore, the quantity of RNA extracted was verified by measuring the concentration of each extraction with a Nanodrop and calculating the total RNA extracted based on the volume. The concentration of each protein extraction was assessed utilizing the bicinchoninic acid (BCA) protein quantification method, and subsequently multiplied by the volume to ascertain the total protein yield.

## 2.9 Non-targeted metabolomics analysis of PnNVs

The PnNVs were subjected to vortexing for a duration of 1 min, followed by the addition of an appropriate sample size to 400 μL of methanol solution. Subsequently, the resulting mixture underwent vortexing for 1 min and was then subjected to centrifugation at 12,000 rpm for 10 min at 4°C. The entirety of the supernatant was collected, concentrated, and subjected to drying. Following the drying process, the sample was reconstituted by the addition of 150 μL of a 2-chloro-L-phenylalanine solution (4 ppm) prepared in 80% methanol in water. The supernatant was subsequently filtered through a 0.22 μm membrane, and the resulting filtrate was transferred to the detection vial for liquid chromatography-mass spectrometry (LC-MS) non-targeted metabolomics analysis (Panomix Biomedical Tech Co., Ltd., Suzhou, China).

## 2.10 Statistical analyses

Numerical results were presented as mean ± standard deviation. Statistical analysis involved the use of t-tests for comparisons



**FIGURE 1** Physical properties of *Panax notoginseng*-derived nanovesicles at different growth stages. (A) Images of *Panax notoginseng* tubers around 35 g in size; (B) Zeta potential of s\_PnNVs; (C) Particle size of s\_PnNVs; (D) Electron microscope images of s\_PnNVs; (E) Images of *Panax notoginseng* tubers around 65 g in size; (F) Zeta potential of m\_PnNVs; (G) Particle size of m\_PnNVs; (H) Electron microscope images of m\_PnNVs; (I) Images of *Panax notoginseng* tubers around 125 g in size; (J) Zeta potential of l\_PnNVs; (K) Particle size of l\_PnNVs; (L) Electron microscope images of l\_PnNVs.

between two groups and one-way Analysis of Variance (ANOVA) for comparisons among three groups. Significant differences identified by ANOVA were further examined through *post hoc* tests for specific group differences.

### 3 Results

#### 3.1 Differences in the physical properties of PnNVs

As previously indicated, PnNVs were categorized into three groups based on the size of *Panax notoginseng* tubers. Specifically, nanovesicles obtained from *Panax notoginseng* tubers weighing approximately 35 g (Figure 1A) were designated as s\_PnNVs, those from tubers weighing around 65 g (Figure 1E) were designated as m\_PnNVs, and nanovesicles isolated from tubers weighing approximately 125 g (Figure 1I) were designated as l\_PnNVs.

The physical characteristics of nanovesicles, including particle size distribution, potential distribution, and morphology, play a significant role in determining their cellular uptake efficiency and subsequent biological activity (Behzadi et al., 2017). In this study, we conducted an evaluation of the fundamental physical properties of PnNVs. The zeta potential of s\_PnNVs was measured at  $-11.78 \pm 0.24$  mV (Figure 1B), with a particle size of  $179.71 \pm 14.46$  nm (Figure 1C). m\_PnNVs exhibited a zeta potential of  $-14.27 \pm$

$2.04$  mV (Figure 1F), with a particle size of  $167.65 \pm 9.86$  nm (Figure 1G). l\_PnNVs had a zeta potential of  $-11.89 \pm 1.07$  mV (Figure 1J), and a particle size of  $191.37 \pm 14.99$  nm (Figure 1K). Through the utilization of transmission electron microscopy, it was observed that all nanovesicles exhibited vesicle-like structures (Figures 1D, H, L). From these findings, it can be inferred that s\_PnNVs possess the least potential, m\_PnNVs have the smallest size, and both m\_PnNVs and l\_PnNVs demonstrate comparable potentials.

#### 3.2 s\_PnNVs exhibit the highest cellular uptake efficiency

The cellular internalization of nanovesicles serve as the foundation for their biological impacts. In order to evaluate variances in their intracellular uptake efficacy of PnNVs, staining and co-localization analyses were conducted. Upon co-incubating DIO dye-labeled PnNVs with LN4 and SCC7 cells for 24 h, it was observed that the green fluorescent signals were notably concentrated within the cells, predominantly localized in the cytoplasm, with the most intense signals observed in the s\_PnNVs group (Figure 2).

The investigation into the efficiency of PnNVs uptake by LN4 cells was conducted using flow cytometry. Results indicated that after 1 h, the fluorescent signals in the cells were minimal and did not exhibit a significant difference (Figures 3A–D).

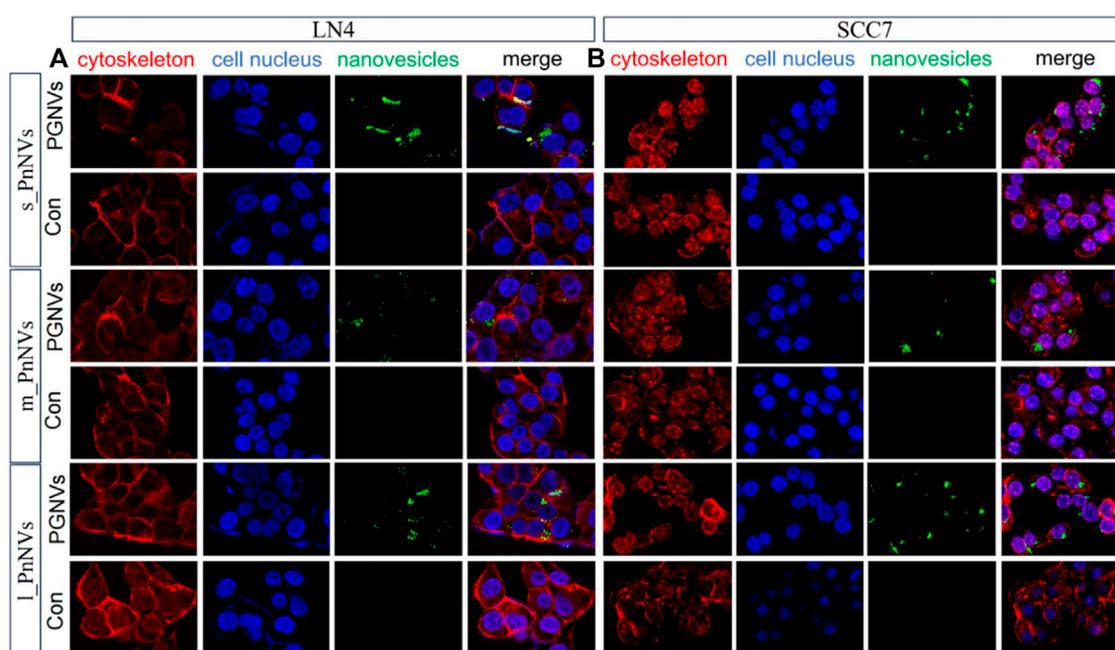


FIGURE 2  
Representative images of cellular uptake of PnNVs. (A) Efficiency of PnNVs uptake by LN4 cells. (B) Efficiency of PnNVs uptake by SCC7 cells.

However, following 12 h of co-incubation, there was a notable increase in fluorescent signals within the LN4 cells (Figures 3E–H), with the s\_PnNVs group showing the highest signal intensity, comprising approximately 33.0% of the total signals (Figure 3E). At the 24-h time point, the fluorescence signal of the s\_PnNVs group remained the most intense, representing approximately 64.7% of the total signals (Figure 3I). Conversely, the signals emitted by m\_PnNVs consistently exhibited lower intensity, accounting for only around 30% of the overall signals at the 24-h time point (Figure 3J). In contrast, l\_PnNVs demonstrated an intermediate uptake efficiency (Figures 3K, L).

Furthermore, the uptake efficiency of PnNVs by SCC7 cells was evaluated. The results indicated that at 1 h, the fluorescent signals within the cells were also faint, with no significant deviation observed compared to the control (Figures 4A–D). Following a 12-h of co-incubation period, the fluorescence signals within SCC7 cells exhibited a significant increase (Figures 4E–H). The s\_PnNVs group demonstrated the highest signal intensity compared to the m\_PnNVs and l\_PnNVs groups, with an enhancement of 55.3% (Figure 4E). Even at the 24-h time point, the s\_PnNVs group maintained the strongest fluorescence signal at approximately 65.9% (Figures 4I–K). Conversely, the m\_PnNVs group were consistently displayed weaker signals, representing only about 25.1% of the total signal at the 24-h time point (Figure 4G). In contrast, the uptake efficiency of l\_PnNVs fell between the other groups at 41.5% (Figures 4K, L).

The aforementioned findings indicate that the uptake efficiency of PnNVs exhibits variability across different cell types. Specifically, s\_PnNVs derived from *Panax notoginseng* at a smaller size demonstrated the highest intracellular uptake efficiency, while m\_PnNVs obtained from *Panax notoginseng* at a medium size exhibited the lowest intracellular uptake efficiency.

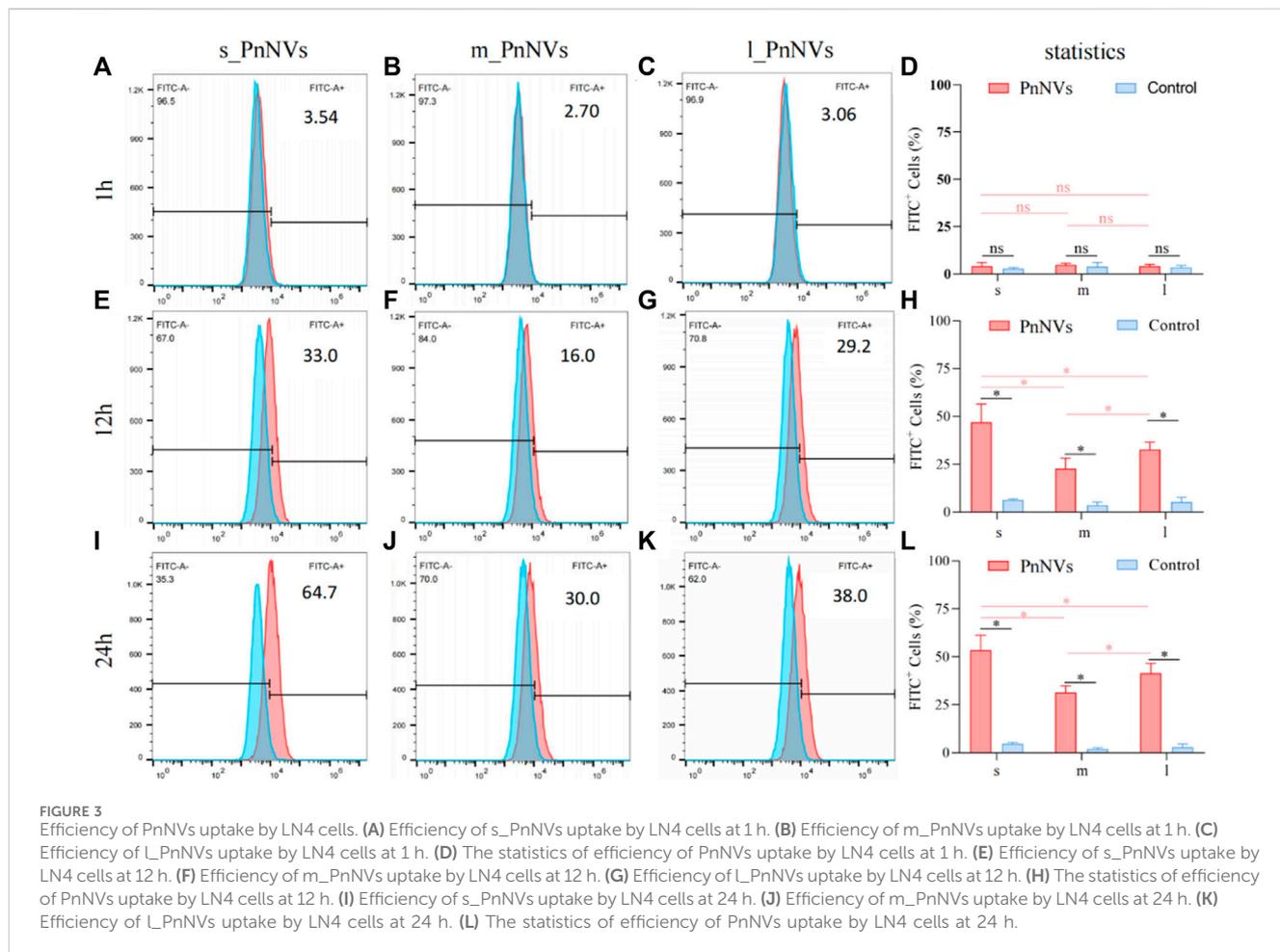
### 3.3 PnNVs were all effective in inhibiting tumor cell proliferation, with m\_PnNVs being the better effectiveness

Malignant proliferation is a prominent characteristic of tumors (Hanahan and Weinberg, 2000). Subsequently, the impact of PnNVs on squamous cells proliferation was investigated. Through CCK-8 assay analysis of cell viability, it was observed that m\_PnNVs displayed greater efficacy in inhibiting the viability of LN4 cells and SCC7 cells (Figure 5). Specifically, at 24 h and 48 h, 12.5  $\mu\text{g}/\text{mL}$  of m\_PnNVs effectively inhibited LN4 cell viability, while s\_PnNVs and l\_PnNVs did not demonstrate inhibitory effects (Figures 5A, E, I). However, at a concentration of 25  $\mu\text{g}/\text{mL}$ , all groups demonstrate effective inhibition cell proliferation viability when exposed to nanovesicles (Figures 5A, E, I).

Subsequent validation experiments conducted with SCC7 cells showed that all groups, except for l\_PnNVs at 12.5  $\mu\text{g}/\text{mL}$ , effectively inhibited cell proliferation viability, as evidenced by the lack of inhibitory effect on cell proliferation viability on the second day (Figures 5C, G, K). Plate cloning experiments, further revealed that 25  $\mu\text{g}/\text{mL}$  of PnNVs successfully inhibited the proliferation of LN4 cells and SCC7 cells, ultimately leading to cell death (Figures 5B, D, F, H, J, L).

### 3.4 PnNVs were all effective in inhibiting tumour cell migration, with m\_PnNVs being the most effectiveness

Metastasis and tumor recurrence are significant contributors to mortality in cancer patients, emphasizing the need for identifying drugs that can impede tumor cell motility (Fares et al., 2020). In this

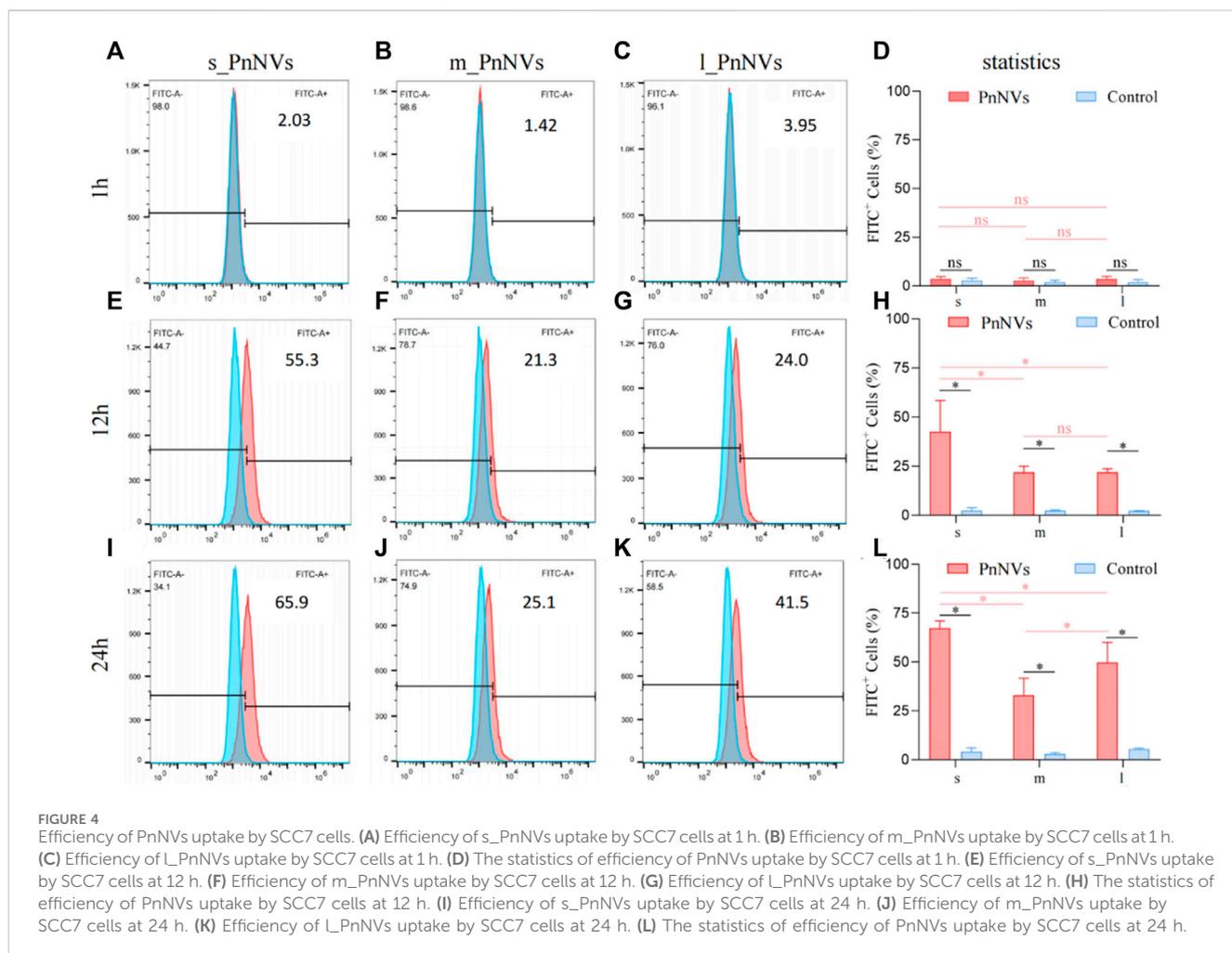


study, we assessed the efficacy of PnNVs in inhibiting the migration of squamous cell carcinoma cells through a scratch assay. Our results demonstrate that PnNVs effectively hindered the scratch healing ability of LN4 and SCC7 cells (Figure 6). Furthermore, statistical analyses revealed that m\_PnNVs outperformed l\_PnNVs in inhibiting cell migration at concentrations of 25  $\mu\text{g}/\text{mL}$  and 50  $\mu\text{g}/\text{mL}$  (Figures 6A, B). However, when conducting individual comparisons between SCC7 cells, no statistically significant differences were observed (Figures 6C, D). These findings indicate that PnNVs possess the capability to hinder cell migration, with m\_PnNVs exhibiting superior outcomes in certain instances.

### 3.5 Compositional analysis of PnNVs

Numerous studies have been conducted on the metabolites of *Panax notoginseng*, with several components demonstrating promising anti-tumor properties (Tan et al., 2021). Additionally, our preliminary analysis identified and quantified RNAs and proteins within these nanovesicles. Our findings indicate that PnNVs contain RNA (Figures 7A–C), although the content did not show significantly variation (Figure 7D). Furthermore, all PnNVs were around to contain proteins (Figures 7E–G), with a higher protein yield observed in nanovesicles of comparable quality from l\_PnNVs (Figure 7H).

The LC-MS analysis of PnNVs metabolites, revealed significant alterations in 52 metabolites in m\_PnNVs compared to s\_PnNVs, with 15 were upregulated and 36 downregulated metabolites (Figures 8A, D). Conversely, when comparing m\_PnNVs to l\_PnNVs, 74 metabolites exhibited changes, with 26 upregulated and 48 downregulated metabolites (Figures 8A, E). Among these altered metabolites, 9,10-Epoxyoctadecenoic acid, Cinobufotalin, Betulin, Stachyose, and Spermidine were identified as common changes, albeit with varying trends (Figure 8B). The metabolite expression levels were found to be lower in m\_PnNVs compared to s\_PnNVs, with these differentially expressed genes showing partial enrichment in tumor-related pathways (Figure 8C). Specifically, only 9,10-Epoxyoctadecenoic acid and Stachyose exhibited lower expression levels in m\_PnNVs compared to l\_PnNVs, while the remaining metabolites displayed higher expression levels. Cinobufotalin (Liu et al., 2019), Betulin (Amiri et al., 2020) and Spermidine (Madeo et al., 2018) are metabolites that have been found to be expressed at elevated levels and possess reported antitumor properties. Additionally, compounds like Rutin (Farha et al., 2022), which is also linked to antitumor effects, were detected in high concentrations. Analysis using the Kyoto Encyclopedia of Genes and Genomes (KEGG) indicated that the identified metabolites are associated with cancer, including central carbon metabolism in cancer, pathways in cancer, renal cell carcinoma, small cell lung cancer. Furthermore, the analysis indicated potential



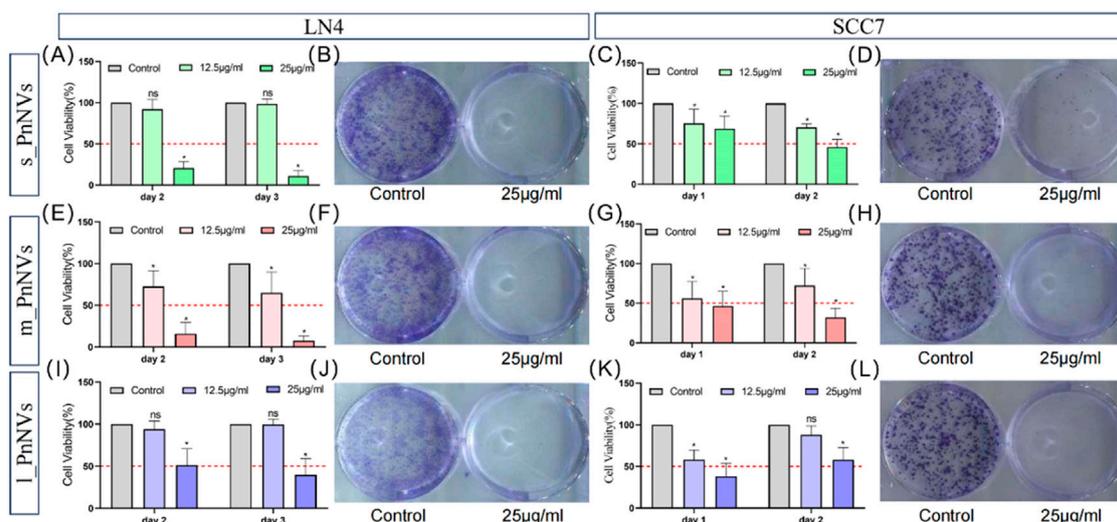
regulation of numerous metabolism-related pathways, such as steroid biosynthesis, TCA cycle, biosynthesis of amino acids, and arginine biosynthesis. Further investigation is warranted to determine whether these regulatory mechanisms contribute to the observed inhibitory effects on tumor cell proliferation and migration. Further investigation is needed (Figures 8C, F). Nevertheless, the precise mechanisms underlying the variations in bioactivity warrant further investigation.

## 4 Discussion

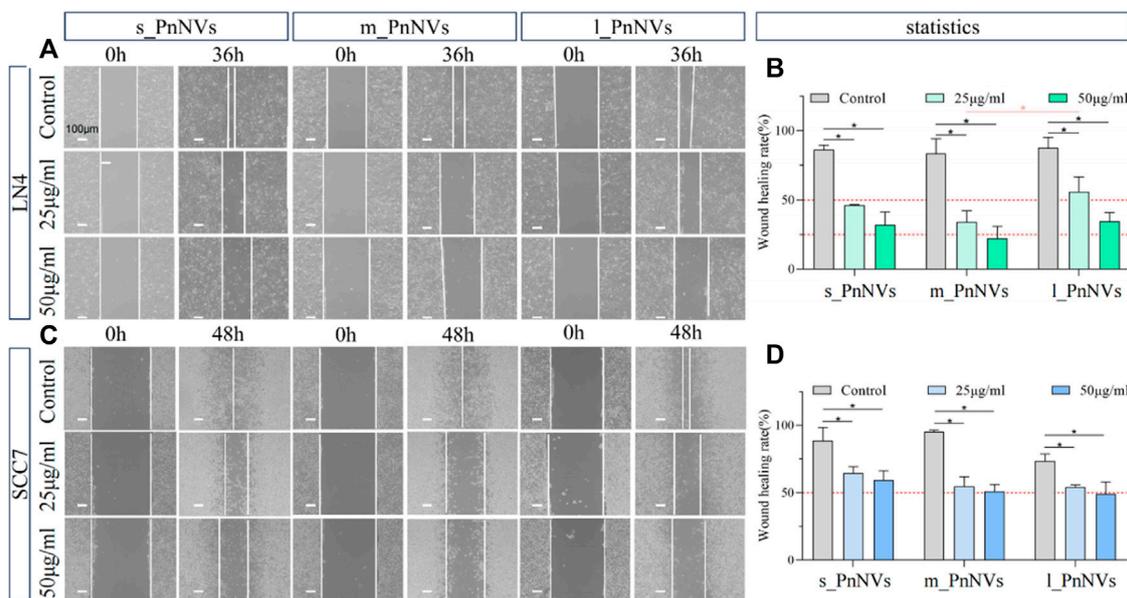
PDNVs are tiny lipid vesicles found in plant cells that are believed to play crucial roles in signaling and substance transport processes within plants (Chen et al., 2023b; Kim and Kim, 2022). There is substantial evidence suggesting that the components present PDNVs possess potential pharmacological activities. For example, studies have shown that messenger RNAs (mRNAs) and small RNAs (sRNAs) contained in PDNVs can regulate the plant immune system and bolster their resistance against pathogens (Cai et al., 2018; Wang et al., 2024). Furthermore, secondary metabolites present PDNVs have been shown to exhibit pharmacological properties, including antioxidant, anti-inflammatory and anti-tumor effects, which could potentially benefit human health (Xu

et al., 2023b). The formation and loading of nanovesicles compositions may be influenced by various factors such as gene expression, growth factors, and environmental conditions. For example, the components encapsulated within nanovesicles may vary depending on the size of the plant tubers, leading to variations in their pharmacological activities. Nevertheless, the mechanisms and patterns governing the sorting of substances within nanovesicles remain poorly understood (Woith et al., 2021; Viršilė et al., 2022).

*Panax notoginseng*, a significant medicinal plant in traditional Chinese medicine (Zhang et al., 2016), is utilized for its therapeutic properties in treating cardiovascular diseases due to its anticoagulant, antihypertensive and vasodilatory attributes. Additionally, *Panax notoginseng* exhibits promising anti-tumor capabilities owing to its antioxidant, anti-inflammatory, and immunomodulatory characteristics (Yang et al., 2018; Xie and Wang, 2023). The primary bioactive constituents of *Panax notoginseng* encompass saponin compounds, polysaccharides, flavonoids and phenolic acids (Liu et al., 2020). The variability of components in *Panax notoginseng* tubers of different sizes can impact their pharmacological activity. Research indicates that various parts of *Panax notoginseng* and its growth stages contain distinct types and quantities of active compounds (Dong et al., 2003). Specifically, saponins are predominant in early growth stages, while polysaccharides and flavonoids are abundant in mature stages. Consequently, variations



**FIGURE 5** Effectiveness of PnNVs in inhibiting cell proliferative activity. (A) Statistical graph of inhibition of LN4 cell viability by different concentrations of s\_PnNVs. (B) Clone formation of LN4 cells after intervention with 25 µg/mL s\_PnNVs. (C) Statistical graph of the inhibition of SCC7 cell viability by different concentrations of s\_PnNVs. (D) Clone formation of SCC7 cells after intervention with 25 µg/mL s\_PnNVs. (E) Statistical graph of inhibition of LN4 cell viability by different concentrations of m\_PnNVs. (F) Clone formation of LN4 cells after intervention with 25 µg/mL m\_PnNVs. (G) Statistical graph of inhibition of SCC7 cell viability by different concentrations of m\_PnNVs. (H) Clone formation of SCC7 cells after intervention with 25 µg/mL m\_PnNVs. (I) Statistical graph of inhibition of LN4 cell viability by different concentrations of l\_PnNVs. (J) Clone formation of LN4 cells after intervention with 25 µg/mL l\_PnNVs. (K) Statistical graph of inhibition of SCC7 cell viability by different concentrations of l\_PnNVs. (L) Clone formation of SCC7 cells after intervention with 25 µg/mL l\_PnNVs.



**FIGURE 6** Effect of PnNVs in inhibiting cell migration activity. (A) Representative images of LN4 cell scratch healing at 0 h and 36 h after intervention with different concentrations of PnNVs. (B) Statistical analysis of the effect of LN4 cell scratch healing at 0 and 36 h after the intervention of different concentrations of PnNVs. (C) Representative images of scratch healing of SCC7 cells at 0 h and 48 h after intervention with different concentrations of PnNVs. (D) Statistical analysis of the scratch healing effect of SCC7 cells at 0 and 48 h after the intervention of different concentrations of PnNVs.

in pharmacological effects may be observed in *Panax notoginseng* of different sizes. The tubers of *Panax notoginseng* are important source of PnNVs and have been identified as containing a high concentration

of active ingredients within these nanovesicles. The active constituents present in PnNVs may play a crucial role in the pharmacological effects of *Panax notoginseng*.

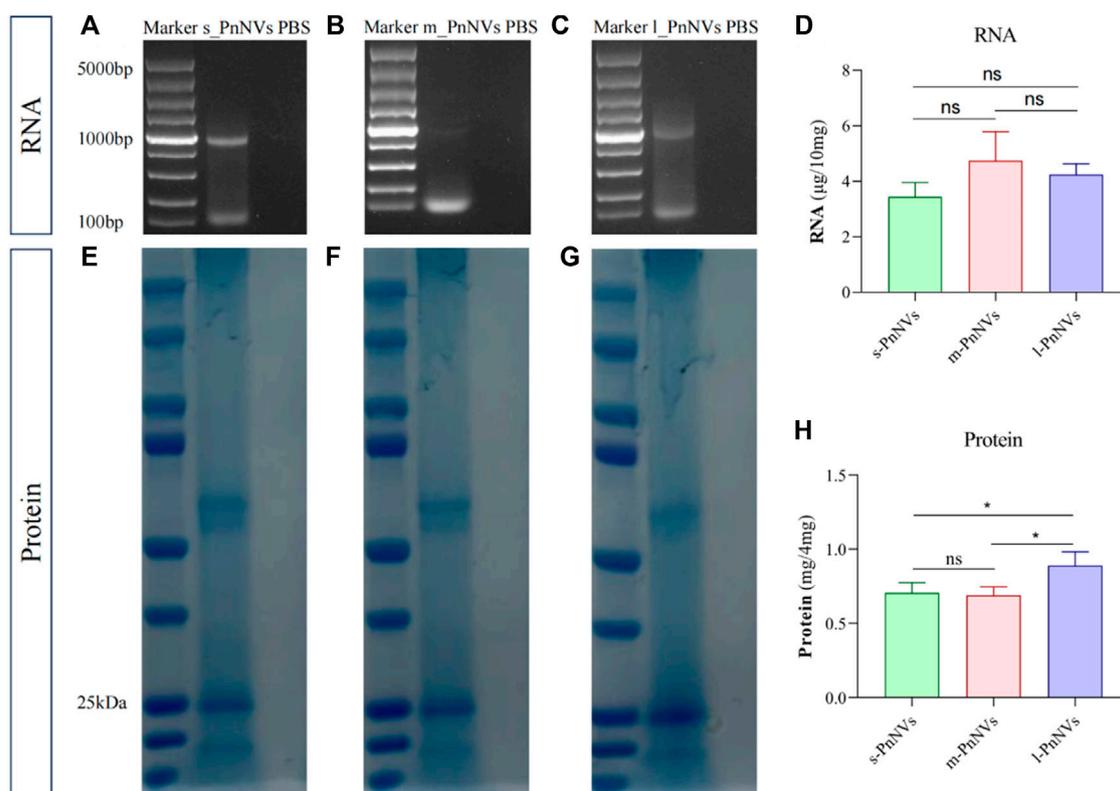


FIGURE 7

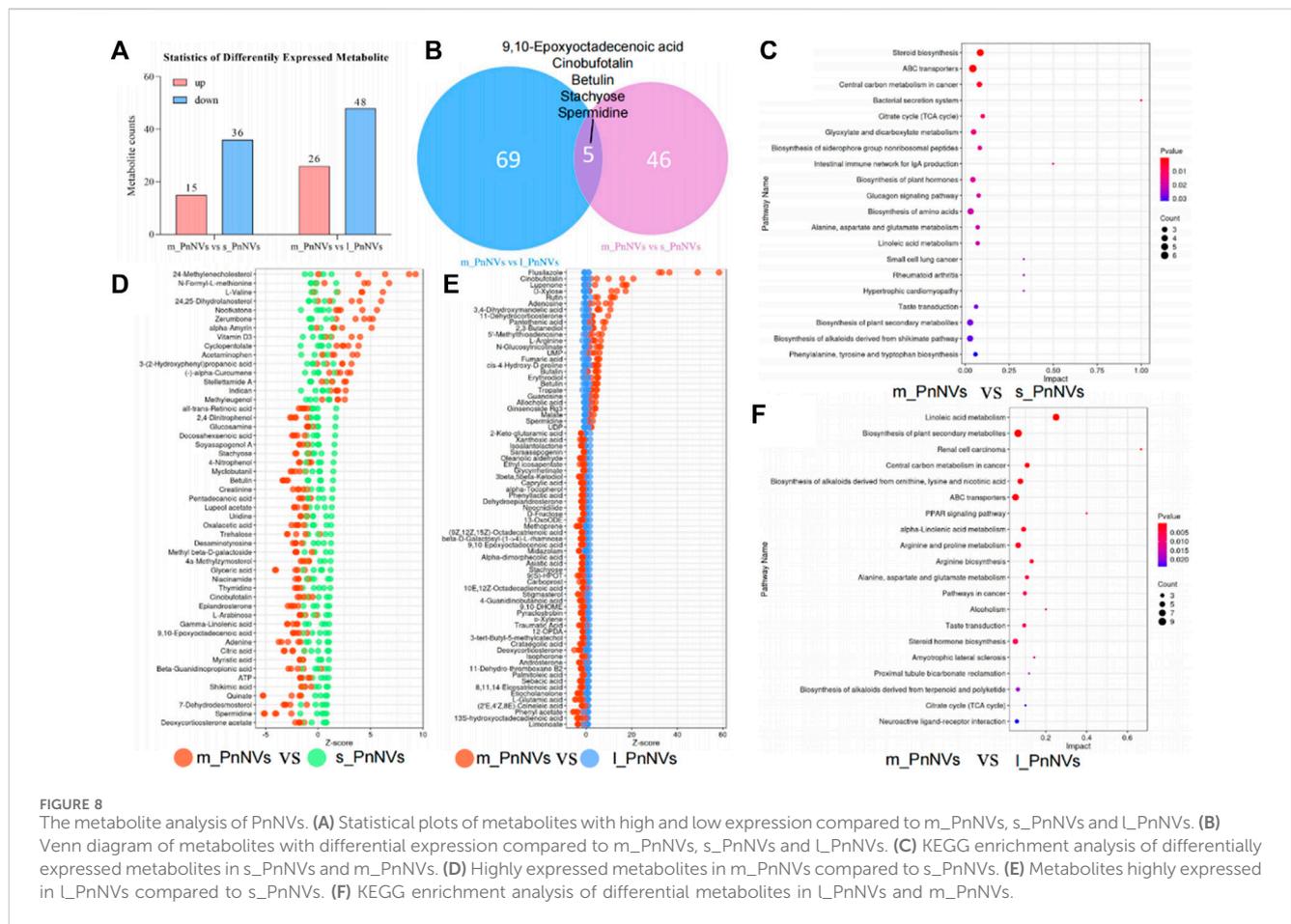
The protein and RNA in PnNVs. (A) Identification plot of RNA isolated from s\_PnNVs (B) m\_PnNVs (C) L\_PnNVs. (D) Amount of RNA isolated in each 10 mg of PnNVs. (E) Identification plots of proteins isolated from s\_PnNVs (F) m\_PnNVs (G) L\_PnNVs. (H) Amount of protein isolated in each 4 mg of PnNVs.

Previous research has demonstrated the potential of PnNVs to mitigate ischemia-reperfusion injury by modulating the polarization state of microglial cells (Li et al., 2023). However, there is a lack of research on the anti-tumor properties of PnNVs. The variability in size of *Panax notoginseng*, a perennial plant, may result in the production of diverse secondary metabolites. The study aims to explore variations in the physical characteristics of nanovesicles produced by *Panax notoginseng* tubers of varying sizes, given their status as a tuberous plant. An analysis of these differences is crucial for understanding the relationship between nanovesicle properties and their source. Furthermore, an evaluation of variances in intracellular uptake efficiency and bioactivity of these nanovesicles could provide valuable insights into their functional mechanisms.

Differences in uptake efficiency of nanovesicles derived from various sources of *Panax notoginseng* were observed in our data, potentially attributed to variations in surface charge and composition of the nanovesicles. The surface charge of nanovesicles plays a crucial role in their interaction with the cell membranes, influencing cellular uptake (Fröhlich, 2012). Our findings indicate that s\_PnNVs exhibited the lowest potential but the highest efficiency in cellular uptake, whereas those m\_PnNVs displayed the highest potential but lowest efficiency in cellular uptake. Additionally, as the presence of proteins and lipids within nanovesicles may influence their intracellular uptake by interacting with cell surface receptors or other cellular

mechanisms (Xu et al., 2022; Zu et al., 2021; Chen et al., 2024). This research has not fully characterized the proteins and other constituents of PnNVs, and further investigation into their components and co-localization analyses is anticipated to elucidate the variations in uptake efficiencies.

Furthermore, metabolomics data indicates a notable abundance of metabolites with strong anticancer properties in m\_PnNVs compared to s\_PnNVs. For example, Nootkatone has been shown to have antiproliferative effects on rectal cancer cells (Yoo et al., 2020), disrupting glucose metabolism, inhibiting the stemness of human breast cancer stem cells (Nguyen et al., 2022), and reducing the sensitivity of tumor cells to certain drugs (Hung et al., 2019). Additionally, Zerumbone (Prasanna et al., 2012), and Methyleugenol (Groh and Esselen, 2017) have been identified as having anti-tumor activity. Importantly, m\_PnNVs exhibit a higher expression of these metabolites with anticancer properties compared to L\_PnNVs. Metabolites such as guanosine (Zhao et al., 2020), rutin (Ghanbari-Movahed et al., 2022), spermidine (Al-Habsi et al., 2022), and cinobufotalin (Han et al., 2021) have demonstrated significant antitumor effects. The metabolites identified in this study exhibited high expression in m\_PnNVs, with Ginsenoside Rg3 (Sun et al., 2022; Zhu et al., 2023), a potent antitumor compound found in *Panax notoginseng*, showing particularly elevated expression. Furthermore, these metabolites were also identified in the KEGG enrichment analysis of pathways related to tumor development. The differential enrichment of these highly expressed metabolites may



play a role in the improved effectiveness of m\_PnNVs, although further research is needed to elucidate the exact mechanisms involved.

It is important to acknowledge that the observed disparity in the size of *panax notoginseng* or ginseng may be attributed to various factors such as the duration of planting, preservation conditions, timing of harvest, as well as levels of dryness and rainfall during the planting phase (Liu et al., 2021b; Tang et al., 2024). This complexity presents a challenge to the study's quality control and necessitates further investigation by experts in the field of agriculture. During the growth of plants, particularly non-perennial species like *Artemisia annua* and dandelions (Thu et al., 2011), the potential variations in their nanovesicles as they progress through germination and flowering in response to seasonal changes warrant further investigation. Additionally, researchers studying herbaceous plants in this area are also concerned with the batch stability of PDNVs.

Despite the initial evaluation of nanovesicles derived from *Panax notoginseng*, it is important to recognize that certain aspects have not been thoroughly examined in the study. One primary limitation is the absence of data derived from animal experiments to support the findings. While existing research has concentrated on squamous cell carcinoma cell models, the response of various cancer cell types or normal cells to PnNVs remains inadequately explored. To address this research gap, future studies could incorporate a diverse range of cell models to investigate the therapeutic efficacy of nanovesicles across different cell types. Such investigations may elucidate the widespread

utility and therapeutic potential of nanovesicles, offering a more foundation of data. Moreover, there is a lack of comprehensive research on the distribution and metabolic kinetics of nanovesicles *in vivo*. Despite the potential of nanovesicles for drug delivery, further detailed investigations are required to elucidate their *in vivo* behavior. This necessitates examinations of the stability of nanovesicles within the circulatory system, their distribution in tissues, and their metabolic pathways. Enhanced knowledge of the *in vivo* behavior of nanovesicles is crucial for maximizing their efficacy in drug delivery and therapeutics applications, and for refining their utilization.

## 5 Conclusion

In summary, the objective of this research was to extract nanovesicles from *Panax notoginseng* tubers of varying sizes and analyze the effects of tuber size on nanovesicles composition, cellular uptake efficiency, and bioactivity. The results indicated noticeable differences in the characteristics of the PnNVs. Notably, s\_PnNVs demonstrated, lowest zeta potential, and the highest cellular uptake efficiency after 24 h. Conversely, m\_PnNVs exhibited the smallest size but the lowest cellular uptake efficiency after 24 h. Notably, PnNVs exhibited pronounced inhibitory effects on the proliferation and migration of LN4 and SCC7 cells, with m\_PnNVs displaying the greatest efficacy. Untargeted metabolomics analysis unveiled a varied compound profile within PnNVs, with metabolites abundantly

expressed in m\_PnNVs being enriched in anti-tumor pathways. Subsequent compositional analysis identified a protein and RNA composition in PnNVs, particularly in l\_PnNVs which contained a higher protein content. In conclusion, our research has presented initial findings indicating that PnNVs derived from varying sizes of *Panax notoginseng* tubers exhibit unique physical characteristics, compositional variances, and effects on cellular activity. These results establish a fundamental basis for further exploration of the therapeutic potential of PnNVs as anti-tumor agents. Nevertheless, further research is warranted to elucidate the biological properties, mechanisms of action, and potential clinical utility of PnNVs.

## Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## Ethics statement

Ethical approval was not required for the studies on humans in accordance with the local legislation and institutional requirements because only commercially available established cell lines were used. Ethical approval was not required for the studies on animals in accordance with the local legislation and institutional requirements because only commercially available established cell lines were used.

## Author contributions

XC: Visualization, Methodology, Data curation, Conceptualization, Writing–review and editing, Writing–original draft. LH: Data curation, Writing–review and editing. MZ: Writing–review and editing. SL: Writing–review and editing. JX: Writing–review and editing. HL: Writing–review and editing. XW: Funding acquisition, Writing–review and editing. YL: Funding

## References

- Al-Habsi, M., Chamoto, K., Matsumoto, K., Nomura, N., Zhang, B., Sugiyama, Y., et al. (2022). Spermidine activates mitochondrial trifunctional protein and improves antitumor immunity in mice. *Science (New York, N.Y.)* 378 (6618), eabj3510. doi:10.1126/science.abj3510
- Amiri, S., Dastghaib, S., Ahmadi, M., Mehrbod, P., Khadem, F., Behrouj, H., et al. (2020). Betulin and its derivatives as novel compounds with different pharmacological effects. *Biotechnol. Adv.* 38, 107409. doi:10.1016/j.biotechadv.2019.06.008
- Behzadi, S., Serpooshan, V., Tao, W., Hamaly, M. A., Alkawareek, M. Y., Dreaden, E. C., et al. (2017). Cellular uptake of nanoparticles: journey inside the cell. *Chem. Soc. Rev.* 46 (14), 4218–4244. doi:10.1039/c6cs00636a
- Cai, Q., Qiao, L., Wang, M., He, B., Lin, F. M., Palmquist, J., et al. (2018). Plants send small RNAs in extracellular vesicles to fungal pathogen to silence virulence genes. *Sci. (New York, N.Y.)* 360 (6393), 1126–1129. doi:10.1126/science.aar4142
- Chen, T., Ma, B., Lu, S., Zeng, L., Wang, H., Shi, W., et al. (2022). Cucumber-derived nanovesicles containing cucurbitacin B for non-small cell lung cancer therapy. *Int. J. Nanomedicine* 17, 3583–3599. doi:10.2147/IJN.S362244
- Chen, X., He, L., Zhang, C., Zheng, G., Lin, S., Zou, Y., et al. (2024). Exploring new avenues of health protection: plant-derived nanovesicles reshape microbial communities. *J. Nanobiotechnology* 22 (1), 269. doi:10.1186/s12951-024-02500-w
- Chen, X., Ji, S., Yan, Y., Lin, S., He, L., Huang, X., et al. (2023b). Engineered plant-derived nanovesicles facilitate tumor therapy: natural bioactivity plus drug controlled release platform. *Int. J. Nanomedicine* 18, 4779–4804. doi:10.2147/IJN.S413831
- Chen, X., Xing, X., Lin, S., Huang, L., He, L., Zou, Y., et al. (2023a). Plant-derived nanovesicles: harnessing nature's power for tissue protection and repair. *J. Nanobiotechnology* 21 (1), 445. doi:10.1186/s12951-023-02193-7
- Dong, T. T., Cui, X. M., Song, Z. H., Zhao, K. J., Ji, Z. N., Lo, C. K., et al. (2003). Chemical assessment of roots of *Panax notoginseng* in China: regional and seasonal variations in its active constituents. *J. Agric. Food Chem.* 51 (16), 4617–4623. doi:10.1021/jf034229k
- Fares, J., Fares, M. Y., Khachfe, H. H., Salhab, H. A., and Fares, Y. (2020). Molecular principles of metastasis: a hallmark of cancer revisited. *Signal Transduct. Target. Ther.* 5 (1), 28. doi:10.1038/s41392-020-0134-x
- Farha, A. K., Gan, R. Y., Li, H. B., Wu, D. T., Atanasov, A. G., Gul, K., et al. (2022). The anticancer potential of the dietary polyphenol rutin: current status, challenges, and perspectives. *Crit. Rev. Food Sci. Nutr.* 62 (3), 832–859. doi:10.1080/10408398.2020.1829541
- Fröhlich, E. (2012). The role of surface charge in cellular uptake and cytotoxicity of medical nanoparticles. *Int. J. Nanomedicine* 7, 5577–5591. doi:10.2147/IJN.S36111
- Gan, R. H., Wei, H., Xie, J., Zheng, D. P., Luo, E. L., Huang, X. Y., et al. (2018). Notch1 regulates tongue cancer cells proliferation, apoptosis and invasion. *Cell Cycle (Georgetown, Tex.)* 17 (2), 216–224. doi:10.1080/15384101.2017.1395534
- Gao, C., Zhou, Y., Chen, Z., Li, H., Xiao, Y., Hao, W., et al. (2022). Turmeric-derived nanovesicles as novel nanobiologics for targeted therapy of ulcerative colitis. *Theranostics* 12 (12), 5596–5614. doi:10.7150/thno.73650

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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- Ghanbari-Movahed, M., Mondal, A., Farzaei, M. H., and Bishayee, A. (2022). Quercetin- and rutin-based nano-formulations for cancer treatment: a systematic review of improved efficacy and molecular mechanisms. *Phytomedicine Int. J. Phytotherapy Phytopharm.* 97, 153909. doi:10.1016/j.phymed.2021.153909
- Groh, I. A. M., and Esselen, M. (2017). Methyl Eugenol and selected oxidative metabolites affect DNA-Damage signalling pathways and induce apoptosis in human colon tumour HT29 cells. *Food Chem. Toxicol. Int. J. Publ. Br. Industrial Biol. Res. Assoc.* 108 (Pt A), 267–275. doi:10.1016/j.fct.2017.08.014
- Han, T., Li, X., Luo, D., Ji, C., Chen, C., and He, C. (2023). Genotype and environment factors driven licorice growth and rhizospheric soil fungal community changes. *Front. Microbiol.* 14, 1308412. doi:10.3389/fmicb.2023.1308412
- Han, Y., Ma, R., Cao, G., Liu, H., He, L., Tang, L., et al. (2021). Combined treatment of cinobufotalin and gefitinib exhibits potent efficacy against lung cancer, evidence-based complementary and alternative medicine. *eCAM* 2021, 6612365. doi:10.1155/2021/6612365
- Hanahan, D., and Weinberg, R. A. (2000). The hallmarks of cancer. *Cell* 100 (1), 57–70. doi:10.1016/s0092-8674(00)81683-9
- Hung, L. V. M., Moon, J. Y., Ryu, J. Y., and Cho, S. K. (2019). Nootkatone, an AMPK activator derived from grapefruit, inhibits KRAS downstream pathway and sensitizes non-small-cell lung cancer A549 cells to adriamycin. *Phytomedicine Int. J. Phytotherapy Phytopharm.* 63, 153000. doi:10.1016/j.phymed.2019.153000
- Kim, J., Zhu, Y., Chen, S., Wang, D., Zhang, S., Xia, J., et al. (2023). Anti-glioma effect of ginseng-derived exosomes-like nanoparticles by active blood-brain-barrier penetration and tumor microenvironment modulation. *J. Nanobiotechnology* 21 (1), 253. doi:10.1186/s12951-023-02006-x
- Kim, S. Q., and Kim, K. H. (2022). Emergence of edible plant-derived nanovesicles as functional food components and nanocarriers for therapeutics delivery: potentials in human health and disease. *Cells* 11 (14), 2232. doi:10.3390/cells11142232
- Li, S., Zhang, R., Wang, A., Li, Y., Zhang, M., Kim, J., et al. (2023). Panax notoginseng-derived exosome-like nanoparticles attenuate ischemia reperfusion injury via altering microglia polarization. *J. Nanobiotechnology* 21 (1), 416. doi:10.1186/s12951-023-02161-1
- Liu, H., Gu, H., Ye, C., Guo, C., Zhu, Y., Huang, H., et al. (2021b). Planting density affects panax notoginseng growth and ginsenoside accumulation by balancing primary and secondary metabolism. *Front. Plant Sci.* 12, 628294. doi:10.3389/fpls.2021.628294
- Liu, H., Lu, X., Hu, Y., and Fan, X. (2020). Chemical constituents of Panax ginseng and Panax notoginseng explain why they differ in therapeutic efficacy. *Pharmacol. Res.* 161, 105263. doi:10.1016/j.phrs.2020.105263
- Liu, J., Wang, Y., Qiu, L., Yu, Y., and Wang, C. (2014). Saponins of Panax notoginseng: chemistry, cellular targets and therapeutic opportunities in cardiovascular diseases. *Expert Opin. Investigational drugs* 23 (4), 523–539. doi:10.1517/13543784.2014.892582
- Liu, J., Xiang, J., Jin, C., Ye, L., Wang, L., Gao, Y., et al. (2023). Medicinal plant-derived mtDNA via nanovesicles induces the cGAS-STING pathway to remold tumor-associated macrophages for tumor regression. *J. Nanobiotechnology* 21 (1), 78. doi:10.1186/s12951-023-01835-0
- Liu, Y., Jiang, Q., Liu, X., Lin, X., Tang, Z., Liu, C., et al. (2019). Cinobufotalin powerfully reversed EBV-miR-BART22-induced cisplatin resistance via stimulating MAP2K4 to antagonize non-muscle myosin heavy chain IIA/glycogen synthase 3 $\beta$ /catenin signaling pathway. *EBioMedicine* 48, 386–404. doi:10.1016/j.ebiom.2019.08.040
- Liu, Y. H., Qin, H. Y., Zhong, Y. Y., Li, S., Wang, H. J., Wang, H., et al. (2021a). Neutral polysaccharide from Panax notoginseng enhanced cyclophosphamide antitumor efficacy in hepatoma H22-bearing mice. *BMC Cancer* 21 (1), 37. doi:10.1186/s12885-020-07742-z
- Ly, N. P., Han, H. S., Kim, M., Park, J. H., and Choi, K. Y. (2023). Plant-derived nanovesicles: current understanding and applications for cancer therapy. *Bioact. Mater.* 22, 365–383. doi:10.1016/j.bioactmat.2022.10.005
- Madeo, F., Eisenberg, T., Pietrocchia, F., and Kroemer, G. (2018). Spermidine in health and disease. *Sci. (New York, N.Y.)* 359 (6374), ean2788. doi:10.1126/science.aan2788
- Nguyen, Y. T., To, N. B., Truong, V. N., Kim, H. Y., Ediriweera, M. K., Lim, Y., et al. (2022). Impairment of glucose metabolism and suppression of stemness in MCF-7/SC human breast cancer stem cells by nootkatone. *Pharmaceutics* 14 (5), 906. doi:10.3390/pharmaceutics14050906
- Niu, W., Xiao, Q., Wang, X., Zhu, J., Li, J., Liang, X., et al. (2021). A biomimetic drug delivery system by integrating grapefruit extracellular vesicles and doxorubicin-loaded heparin-based nanoparticles for glioma therapy. *Nano Lett.* 21 (3), 1484–1492. doi:10.1021/acs.nanolett.0c04753
- Prasanna, R., Kalesh, K. A., Shanmugam, M. K., Nachiyappan, A., Ramachandran, L., Nguyen, A. H., et al. (2012). Key cell signaling pathways modulated by zeranolone: role in the prevention and treatment of cancer. *Biochem. Pharmacol.* 84 (10), 1268–1276. doi:10.1016/j.bcp.2012.07.015
- Sun, D., Zou, Y., Song, L., Han, S., Yang, H., Chu, D., et al. (2022). A cyclodextrin-based nanoformulation achieves co-delivery of ginsenoside Rg3 and quercetin for chemo-immunotherapy in colorectal cancer. *Acta Pharm. Sin. B* 12 (1), 378–393. doi:10.1016/j.apsb.2021.06.005
- Tan, M. M., Chen, M. H., Han, F., Wang, J. W., and Tu, Y. X. (2021). Role of bioactive constituents of panax notoginseng in the modulation of tumorigenesis: a potential review for the treatment of cancer. *Front. Pharmacol.* 12, 738914. doi:10.3389/fphar.2021.738914
- Tang, J., Yang, Q., Liang, J., Wang, H., and Yue, X. (2024). Water management, planting slope indicators, and economic benefit analysis for Panax notoginseng production decision under shaded and rain-shelter cultivation: a three-year sloping fields experiment. *Agric. Water Manag.* 291, 108635. doi:10.1016/j.agwat.2023.108635
- Thu, B. T., Van Minh, T., Lim, B. P., and Keng, C. L. (2011). Effects of environmental factors on growth and artemisinin content of artemisia annua L. *Trop. Life Sci. Res.* 22 (2), 37–43.
- Viršilė, A., Samuolienė, G., Laužikė, K., Šipailaitė, E., Balion, Z., and Jekabsone, A. (2022). Species-specific plant-derived nanoparticle characteristics. *Plants (Basel, Switzerland)* 11 (22), 3139. doi:10.3390/plants11223139
- Wang, P., Cui, J., Du, X., Yang, Q., Jia, C., Xiong, M., et al. (2014). Panax notoginseng saponins (PNS) inhibits breast cancer metastasis. *J. Ethnopharmacol.* 154 (3), 663–671. doi:10.1016/j.jep.2014.04.037
- Wang, S., He, B., Wu, H., Cai, Q., Ramírez-Sánchez, O., Abreu-Goodger, C., et al. (2024). Plant mRNAs move into a fungal pathogen via extracellular vesicles to reduce infection. *Cell Host Microbe* 32 (1), 93–105.e6. doi:10.1016/j.chom.2023.11.020
- Woith, E., Guerriero, G., Hausman, J. F., Renaut, J., Leclercq, C. C., Weise, C., et al. (2021). Plant extracellular vesicles and nanovesicles: focus on secondary metabolites, proteins and lipids with perspectives on their potential and sources. *Int. J. Mol. Sci.* 22 (7), 3719. doi:10.3390/ijms22073719
- Xiao, Q., Zhao, W., Wu, C., Wang, X., Chen, J., Shi, X., et al. (2022). Lemon-derived extracellular vesicles nanodrugs enable to efficiently overcome cancer multidrug resistance by endocytosis-triggered energy dissipation and energy production reduction. *Adv. Sci. (Weinheim, Baden-Wuerttemberg, Germany)* 9 (20), e2105274. doi:10.1002/advs.202105274
- Xie, Y., and Wang, C. (2023). Herb-drug interactions between Panax notoginseng or its biologically active compounds and therapeutic drugs: a comprehensive pharmacodynamic and pharmacokinetic review. *J. Ethnopharmacol.* 307, 116156. doi:10.1016/j.jep.2023.116156
- Xu, F., Mu, J., Teng, Y., Zhang, X., Sundaram, K., Sriwastva, M. K., et al. (2022). Restoring oral nanoparticles mediated brain memory function of mice fed alcohol by sorting inflammatory dectin-1 complex into microglial exosomes. *Small (Weinheim an der Bergstrasse, Germany)* 18 (6), e2105385. doi:10.1002/sml.202105385
- Xu, X., Deng, G., Li, X., Li, P., Chen, T., Zhou, L., et al. (2023a). Extraction, structural, and antioxidant properties of oligosaccharides hydrolyzed from panax notoginseng by ultrasonic-assisted fenton degradation. *Int. J. Mol. Sci.* 24 (5), 4506. doi:10.3390/ijms24054506
- Xu, Z., Xu, Y., Zhang, K., Liu, Y., Liang, Q., Thakur, A., et al. (2023b). Plant-derived extracellular vesicles (PDEVs) in nanomedicine for human disease and therapeutic modalities. *J. Nanobiotechnology* 21 (1), 114. doi:10.1186/s12951-023-01858-7
- Yang, B. R., Yuen, S. C., Fan, G. Y., Cong, W. H., Leung, S. W., and Lee, S. M. (2018). Identification of certain Panax species to be potential substitutes for Panax notoginseng in hemostatic treatments. *Pharmacol. Res.* 134, 1–15. doi:10.1016/j.phrs.2018.05.005
- Yang, M., Luo, Q., Chen, X., and Chen, F. (2021). Bitter melon derived extracellular vesicles enhance the therapeutic effects and reduce the drug resistance of 5-fluorouracil on oral squamous cell carcinoma. *J. Nanobiotechnology* 19 (1), 259. doi:10.1186/s12951-021-00995-1
- Yoo, E., Lee, J., Lertpatipatpong, P., Ryu, J., Kim, C. T., Park, E. Y., et al. (2020). Anti-proliferative activity of A. Oxyphylla and its bioactive constituent nootkatone in colorectal cancer cells. *BMC Cancer* 20 (1), 881. doi:10.1186/s12885-020-07379-y
- Zhang, H. Z., Liu, D. H., Zhang, D. K., Wang, Y. H., Li, G., Yan, G. L., et al. (2016). Quality assessment of panax notoginseng from different regions through the analysis of marker chemicals, biological potency and ecological factors. *PLoS One* 11 (10), e0164384. doi:10.1371/journal.pone.0164384
- Zhang, P., Tang, R., Yang, S., Jiang, D., Yuan, M., Li, H., et al. (2022). Preparation and *in vitro* release of mPEG-PLA microspheres of Panax notoginseng saponins. *Int. J. Biol. Macromol.* 217, 922–930. doi:10.1016/j.ijbiomac.2022.07.195
- Zhao, H., Feng, H., Liu, J., Tang, F., Du, Y., Ji, N., et al. (2020). Dual-functional guanosine-based hydrogel integrating localized delivery and anticancer activities for cancer therapy. *Biomaterials* 230, 119598. doi:10.1016/j.biomaterials.2019.119598
- Zheng, Y. R., Fan, C. L., Chen, Y., Quan, J. Y., Shi, L. Z., Tian, C. Y., et al. (2022). Anti-inflammatory, anti-angiogenic and antiviral activities of dammarane-type triterpenoid saponins from the roots of Panax notoginseng. *Food and Funct.* 13 (6), 3590–3602. doi:10.1039/d1fo04089h
- Zhu, Y., Wang, A., Zhang, S., Kim, J., Xia, J., Zhang, F., et al. (2023). Paclitaxel-loaded ginsenoside Rg3 liposomes for drug-resistant cancer therapy by dual targeting of the tumor microenvironment and cancer cells. *J. Adv. Res.* 49, 159–173. doi:10.1016/j.jare.2022.09.007
- Zhu, Z., Gui, Y., Wang, L., Wang, T., Yang, Y., Niu, Y., et al. (2017). Innovative development path of ethnomedicines: a case study. *Front. Med.* 11 (2), 297–305. doi:10.1007/s11684-017-0513-z
- Zu, M., Xie, D., Canup, B. S. B., Chen, N., Wang, Y., Sun, R., et al. (2021). Green nanotherapeutics from tea leaves for orally targeted prevention and alleviation of colon diseases. *Biomaterials* 279, 121178. doi:10.1016/j.biomaterials.2021.121178