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NANOVESICLES FROM PLANTS AS EDIBLE CARRIERS OF BIOACTIVE COMPOUNDS

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Abstract

Nanosized vesicles are released by animal cells in the extracellular environment and have been retrieved in body fluids. Their small dimensions and relative stability as compared to synthetic liposomes has prompted their use as drug and gene delivery vehicles. However, the use of animal vesicles originating from cultured cells is hampered by safety issues. Recent findings have shown that edible plant-derived nanovesicles with a biochemical content resembling that of vesicles isolated from animal cells and body fluids could be obtained from vegetal sources, such as grape and grapefruit juices. These nanovesicles improved the stability and bioavailability of orally administered bioactive compounds such as curcumin and have been proposed as a therapeutic approach for the treatment of cancer and immunological disorders of the digestive tract. Furthermore, they have also shown therapeutic efficacy by themselves, being able to stimulate signaling pathways in intestinal target cells and demonstrating a cross-kingdom ability to transmit signals between vegetal and animal cells, that foster their use as nutriceuticals.

Key words*: plant edible nanovesicles, exosomes, drug delivery, therapeutic carriers, nutriceuticals.*

INTRODUCTION

Animal cells release in the extracellular environment membrane surrounded extracellular vesicles (EVs) of different subcellular origin and dimensions. Most of these vesicles originate either from the outward budding of the plasma membrane (microvesicles or ectosomes) or are Intraluminal vesicles (ILVs) contained in Multivesicular Bodies (MVBs) of the late endosomal compartment, which are released outside the cell upon fusion of the MVB with the plasma membrane (exosomes). Microvesicles are characterized by heterogenous shape and dimensions ranging from 100 nm to 1000 nm, whereas exosomes are smaller, with a diameter between 30 nm and 120 nm (Urbanelli et al., 2013). After being released extracellularly, EVs end up in biological fluids and over the last 30 years, they have been isolated from many sources, namely blood, urine, saliva, cerebrospinal and amniotic fluid, breast milk (Yanez Mo et al., 2015). From a physiological point of view, they have been initially considered as disposal bags for unnecessary substances, but it has later emerged that they actually have important functions in cell-to cell communication and must be considered an additional manner to transmit signals outside the cell, even in distant districts of the body and across biological barriers. EVs have a peculiar and complex biochemical composition, which is reminiscent of the cell of origin. In addition to lipids and proteins, they have raised considerable interest because they contain nucleic acids, namely mRNA and miRNA. To this regard, they have also been demonstrated to be a vehicle for horizontal gene transfer, as after transfer of exosomal mRNA, new proteins were found in the recipient cells, indicating that transferred exosomal mRNA are translated (Valadi et al., 2007).

The size, biochemical features and widespread distribution of EVs have suggested many potential applications (Urbanelli et al., 2015). From a therapeutic point of view, EVs are able to present antigens and have been employed as immunostimulatory agents. More recently, it has been demonstrated that many regenerative properties of stem cells can be mimicked by stem cell released EVs, thus fostering their use in regenerative medicine. The presence of EVs

in easily accessible circulating biofluids has prompted their use for diagnosis and prognostic purposes, in particular as biomarkers in cancer.
Furthermore, another property has attracted the interest of the scientific community for EVs: they are relatively stable in biological fluids, i.e. much more resistant to degradation than synthetic liposomes, and their surface can be modified to increase their ability to target specific tissues or pass important barriers. This feature has suggested their use as drug delivery carriers for small molecules as well as for nucleic acids. As a matter of fact, EVs are nanovectors that protect their own nucleic acid content from degradation in biological fluids. EVs have been successfully loaded with many important drugs, such as paclitaxel, doxorubicin and methotrexate or with nucleic acids such as siRNA and miRNA (Pérez-Bermúdez et al., 2017), showing the ability to increase the stability and bioavailability of these therapeutic agents in vivo.
Nevertheless, some problems related to the use of EVs as drug delivery carriers remain. Isolation and characterization procedures are far from being standardized and although safety risks for EVs are reduced with respect to cell-based therapies, their use require standard preparation protocols and imply regulatory issues that must be addressed. This evidence has prompted the search for a safer source of EVs for therapeutic purposes. A possible approach is the use of EVs synthetic mimetics, i.e. liposome with a lipid composition and surface charge based on the lipid composition and surface charge of EVs. However, the recent isolation of nanovesicles (NVs) in edible plants with structure and composition that appear comparable to mammalian cell-derived small EVs, i.e. exosomes, has opened a new perspective for the therapeutic application of EVs as nanocarriers.

NANOVESICLES FROM EDIBLE PLANTS

Recent studies have shown in plants the exocytosis of NVs structurally resembling mammalian exosomes. Plant MVBs implicated in the release of anti-microbial compounds to fight against fungal infections and to help fertilization have been described (Yañez-Mó et al., 2015). Moreover, there is evidence that vesicles may be involved not only in plant-cell communication but also in cross-kingdom communication between plants and animals: a plant-derived miRNA such as miR-168 from rice has been reported to enter the circulation of rice-fed mice enclosed in vesicles (Zhang et al., 2012).
Currently, NVs have been isolated from different edible plants (grapefruits, grapes, ginger, carrots and citrus limon) and their structure and function have been characterized (Ju et al., 2013; Mu et al., 2014; Wang et al., 2014; Raimondo et al., 2015). Since they are round-shaped and have a structure closely related to mammalian exosomes, they have been often called exosome-like nanovesicles or exosome-like nanoparticles.
Plant-derived NVs have been usually isolated by differential centrifugation followed by a density gradient centrifugation step using plant juices as starting material, a protocol already used for the isolation of mammalian exosomes. Biophysical approaches (electron microscopy, nanoparticle tracking analysis and dynamic light scattering) have shown that these nanovesicles are exosome-like structures whose average diameter depends on the vegetal source. In fact, the diameter of nanovesicles from lemon juice was between 50 and 70 nm (Raimondo et al., 2015), whereas NVs with a larger diameter were observed in grape (~400 nm), grapefruit and ginger (~250 nm) (Ju et al., 2013; Wang et al., 2014; Mu et al., 2014).
The size of NVs is an important parameter as it influences their interaction with recipient cells, namely with intestinal mucus cells (Zhang et al., 2016). As mammalian exosomes, plant-derived NVs usually show negative surface charge (-49.2 to 1.52 mV zeta potentials), indicating that they exhibit mutual repulsion and no tendency toward aggregation (Zhang et al., 2016).
Further analyses have been also carried out to test the stability of plant-derived NVs in stomach-like and intestinal-like conditions by resuspending the NVs either in water or in stomach-like and intestinal-like solutions, then analysing their size and surface charge. Results have demonstrated that the size and charge of the plant-derived NVs are modified in pH-dependent manner (Mu et al., 2014; Wang et

al., 2014), but the modification of these biophysical parameters depends on the vegetable source. Mu and coworkers (2014) showed that grape-derived NVs became smaller in size in stomach-like and intestine-like solutions, whereas in the case of ginger NVs, a subset of the population enlarged in size was generated in stomach-like and intestinal-like solutions. The size distribution of NVs derived from grapefruit and carrots was modified in stomach-like solution, but not in intestinal-like solution. pH-depended changes of NVs surface charge were also observed (Mu et al., 2014; Wang et al., 2014). Specifically, the surface charge of the NVs derived from different vegetal sources was still negative in intestinal-like solution whereas a remarkable reduction in negative charge was observed in stomach-like solution (Mu et al., 2014; Wang et al., 2014). Wang and coworkers (2014) also evaluated the stability of grapefruit-derived NVs after serial digestion in gastric and intestinal enzymatic solutions and demonstrated that these NVs are resistant to digestion by both gastric and intestinal solution.

The biochemical composition of plant-derived NVs shows peculiarities with respect to mammalian exosomes and is strictly dependent on the vegetable origin. For this reason, it is not surprising that NVs from different plants have different biological effects on recipient mammalian cells (Mu et al., 2014). Firstly, plant-derived NVs contain RNA and specifically a substantial amount of small-sized RNAs, thus indicating that plant-derived NV preparations are enriched in exosome-like nanoparticles resembling animal exosomes (Ju et al., 2013; Mu et al., 2014). NVs miRNA profile indicated that vesicles contain miRNA and specifically grape-derived NVs contain miRNA enriched for the miRNA169 family (Ju et al., 2013).

Proteomic analyses allowed to identify 28 proteins in NVs from grapes (Ju et al., 2013) and 137 proteins in NVs from grapefruit (Wang et al., 2014). The protein profile indicated that a number of these proteins regulate carbohydrate and lipid metabolism (Wang et al., 2014). Interestingly, in the apoplastic fluid of sunflower a protein showing 68% identity with human Rab11a GTPase (a mammalian exosome protein) has been identified, indirectly confirming the similarities between plant and mammalian nanovesicles (Yañez-Mó M. et al., 2015).

Lipidomic analyses demonstrated differences in lipid composition of NVs of different origin. Like mammalian exosomes, grapefruit NVs are enriched in PE (~ 45%) and PC (~ 28%); in particular, they are rich of PE (34:2) and PC (34:2) molecular species (Wang et al., 2014). A peculiar lipid composition was observed in grape NVs that are enriched in PA (~53%) and PE (~26%) (Ju et al., 2014).

PLANT-DERIVED NANOVESICLES AS THERAPEUTIC TOOLS

Studies reported in the previous section not only investigated the structure and the biological composition of plant-derived NVs, but also determined their biological effect on mammalian cells.

Results demonstrated that these nanoparticles are taken up by intestinal cells and influence intestinal regeneration and anti-inflammatory responses (Ju et al., 2013; Mu et al., 2014; Wang et al., 2014). The first demonstration that plant-derived NVs can penetrate the intestinal mucus barrier derived from the study of Ju and coworkers (2013). This study showed that grape NVs are taken up by mouse intestinal stem cells and subsequently strongly promote the proliferation of intestinal stem cells through the Wnt/β-catenin pathway. In addition, it has been provided evidence that the oral administration of NVs leads to protection of mice from dextran sulphate sodium (DSS)-induced colitis via induction of intestinal stem cells proliferation. NV lipids seems play an important role in targeting intestinal stem cells and in the promotion of their proliferation.

Mu and coworkers (2014) showed that NVs isolated from four edible plants (ginger, carrot, grape and grapefruit) are taken up by intestinal macrophages and stem cells and exerted biological effects acting on different intracellular targets.

The four plant-derived NVs induced in macrophages the activation of anti-inflammatory and anti-oxidant processes, whereas only ginger NVs were able to induce

the expression of the antioxidation gene heme oxygenase-1 and of the anti-inflammatory cytokine IL-10. Furthermore, all the different NVs considered in this paper induces an activation of Wnt signalling, which is crucial for maintaining intestinal homeostasis.

Recently a role of plant-derived NVs in influencing cancer progression has been demonstrated. In this regard, Raimondo and coworkers (2015) showed that NVs from citrus lemon juice inhibit cell proliferation in different tumor cell lines by activating TRAIL-mediated apoptotic cell death. Authors also demonstrated, using an *in vivo* xenograft model of chronic myeloid leukemia, that citron lemon NVs reached the tumor site and suppressed tumor growth by activating TRAIL-mediated apoptotic cell processes and inhibiting of angiogenic processes.

The identification of exosome-like nanoparticles in plants, with a structure and composition not distant from that of mammalian derived exosomes, has suggested their use as an alternative tool for drug and gene delivery. This approach is based on vesicles prepared from edible plants, and shows evident benefits in terms of safety and cost. Vesicles isolated from fruit juices (e.g., grape, grapefruit, and tomatoes) or from Actaea species have been loaded with different therapeutic drugs for the treatment of cancer and inflammatory disorders (Urbanelli et al., 2015). In two studies, it was also demonstrated that chemotherapeutic drugs such as methotrexate as well as siRNAs can be encapsulated into plant NVs (Wang et al., 2013; Wang et al, 2014). More recently, the capability of a grapefruit-derived nanovector to carry miR-17 for therapeutic treatment of mouse brain tumor has also been shown, providing the proof of principle that these vesicles can be used to deliver a miRNA across the blood–brain barrier via an unconventional route, i.e. intranasal delivery (Zhuang et al., 2016).

In the case of curcumin, its improved stability and bioavailability once encapsulated in plant NVs has led to ongoing clinical trials investigating the ability of such nanocarriers to deliver curcumin to normal and colon cancer tissue (https:// clinicaltrials.gov/ct2/show/ NCT01294072).

CONCLUSIONS

The isolation of nanovesicles from plants suggest many relevant applications. Nanovesicles from plant appear to communicate with the entire digestive tracts of mammals and mediate important physiological functions, representing a tool for cell communication between plants and animals. Their structural and biochemical features will prompt their therapeutic use not only as carriers for bioactive compounds and nutriceuticals, but also as drug delivery vehicles, that can be loaded with drugs or genes to improve the stability and bioavailability of these therapeutics.

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