Editorial Commentary

Extracellular vesicle-mediated transfer of mitochondria between organs

Alice Cristina Rodrigues

Department of Pharmacology, Universidade de Sao Paulo Instituto de Ciencias Biomédicas, São Paulo, Brazil

Correspondence to: Alice Cristina Rodrigues, PhD. Department of Pharmacology, Universidade de Sao Paulo Instituto de Ciencias Biomédicas, 05508-000 Sao Paulo, Brazil. Email: alice-rodrigues@usp.br.

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The term extracellular vesicle (EV) is the consensus generic term for lipid bilayer-delimited particles released from the cell that cannot replicate (1). All cells, prokaryotes and eukaryotes, release EVs as part of their normal physiology and under acquired abnormalities. Generally, EVs can be divided into two categories: endosome-origin exosomes or plasma membrane—derived ectosomes (microvesicles). Exosomes are small vesicles, ranging from 30–150 nm, generated by the fusion of multivesicular bodies (MVBs). Ectosomes are larger EVs in the size of 50–1,000 nm that bud off from the plasma membrane. Nevertheless, no consensus has yet emerged on specific markers of subcellular origin and currently EV identity is classified and characterized based on physical characteristics (size/density), biochemical composition and description of conditions or cell of origin as a replacement for terms exosome and microvesicle.

The existence of EVs is not an entirely new concept and the term was first used in 1971 by Aaronson *et al.* (2), who clearly recognized EV biogenesis as a biological phenomenon. Although the MVB was first described in 1955 (3), the term exosome to refer to EVs was not used until 1981 by Trams *et al.* (4). However, only in 1987, after a paper of Johnstone and colleagues (5) describing that during reticulocyte maturation surface membrane components are released in vesicular form, consensus quickly developed around the use of exosome to describe endosome-origin EVs.

Originally disregarded as simple vesicles, with further study and increasing consciousness of the role and function of EV, now they are recognized to play important roles in many physiological and pathological processes.

EVs have been shown to be mediators of intercellular communication as they can transfer their cargo from a donor cell to a recipient cell. EVs contain a number of cargo components, such as proteins, lipids and DNA and different classes of RNAs. Their composition depends largely on their cellular origins and on the functional states of cells producing them, that is, whether they are rested, stimulated, transformed, or stressed (6). The encapsulated cargoes of EV are protected from degradation and stably maintained for long-distance transport. Therefore, transport through EV is an effective way for surroundings and distant cells to communicate.

As mentioned before, EVs are released in the extracellular environment from cells upon fusion of MVB with the plasma membrane or secreted from direct budding from the plasma membrane of the host cell. Following their release in the milieu, EV can interact with recipient cells by at least three mechanisms: (I) direct fusion with the plasma membrane of the recipient cells; (II) receptor-mediated endocytosis; (III) protein-protein or receptor-ligand interactions between EV and recipient cell surface, which can initiate signaling cascades that activate endocytic pathway.

Besides mediating paracrine communication, it was firstly demonstrated by Dr. Kahn group that EVs can mediate endocrine communication between different tissues (7). They elegantly demonstrated that EV-derived microRNAs are released from adipose tissue to modulate gene expression in the liver. Even though it is unclear which

cell type contributes the majority of EVs to circulation, the Thomou *et al.* (7) study suggests adipocytes EVs can contribute to whole-body metabolic signaling and more importantly that adipocyte communicate with other organs beyond adipokines, signaling lipids and nutrients.

EVs cargoes reflect the host cells activity, thus, their levels could also change in diseases with altered fat mass, such as lipodystrophy and obesity, or altered adipose distribution and function, such as diabetes and ageing. Indeed, it has been shown that circulating EV from diet-induced obesity individuals have a different content of microRNAs compared to lean subjects and losing weight by exercising only can reestablish their levels (8). Moreover, obesity increases the content of EV in circulation and circulating EVs are involved in the development of obesity-associated glucose intolerance and dyslipidemia in mice (9).

Obesity, an abnormal or excessive expansion of adipose tissue, is associated with a diversity of comorbidities, including diabetes type 2 and cardiovascular diseases (10,11). In the context of obesity-related cardiovascular diseases, it has been explained through the impact of low-grade inflammation, increased oxidative stress and the associated cardio metabolic alterations (12,13). Ectopic fat deposition in the heart also is a hallmark of adipose tissue dysfunction and a trigger of most of the damages promoted by obesity in the cardiac tissue (14,15).

Recently, a group of investigators developed a transgenic model of obesity that provides an opportunity to study the role of adipose tissue in the development of comorbidities (16). In this model, mitochondrial ferritin (FtMT) is overexpressed specifically in adipose tissue (adipo-FtMT mouse) promoting mitochondrial dysfunction in adipocytes. Interestingly, cardiac tissue of these mice is highly susceptible to oxidative stress.

In a recent issue of Cell Metabolism, an exciting new paper by Crewe *et al.* (17) revealed that EVs released by adipocytes can mediate increased oxidative stress in the heart of adipo-FtMT mouse model. Evidence is provided that adipocytes undergoing mitochondria-specific stress release small EVs containing mitochondrial fragments that are taken up by cardiomyocytes.

Adipo-FtMT mice on a high-fat diet with doxycycline (dox-HFD) had higher levels of protein carbonylation compared to controls on the same diet. The detrimental effect of feeding a dox-HFD for a short period (3 weeks) was similar to that observed in the heart of wild-type mice after 16 weeks of HFD compared to their respective controls (17). Next, the researchers tested the possible involvement of EVs as mediators of the observed crosstalk between adipocytes and cardiomyocytes. Adipo-FtMT had increased levels of circulating EVs than control mice, an effect common to other mouse models of mitochondrial dysfunction. Inhibition of neutral sphingomyelinase (n-Smase) with acute injection of GW4869 completely abolished the rise in serum EVs in adipo-FtMt mice and more importantly, fully prevented the induction of cardiac oxidative stress. Further proteomics analysis revealed that the pro-oxidant signals carried by EVs from adipocytes to the heart are fragments of mitochondria, reminiscent of intracellular vesicles called mitochondrial-derived vesicles (MDVs).

MDVs are generated through the selective incorporation of protein cargoes, which can be limited to the outer membrane, or can include outer, inner membrane, and matrix content (18-20). MDV transport to lysosomes has been proposed as a new pathway for mitochondrial quality control (21). However, MDV can escape from lysosomal degradation by release via EVs as suggested previously by Sugiura and recently demonstrated (17,22). Noteworthy, Crewe *et al.* (17) have shown that mitochondrial content in EV is functionally significant and mitochondrial transfer between distant tissues constitute a previously undescribed mechanism by which adipocytes can communicate with distant tissues.

The mechanism underlying the etiopathogeny of obesity-associated cardiovascular disease is not fully understood. The discovery of EVs as new mediators opens the possibility that a new range of engineered EVs containing specific cargoes are designed to deliver the content to the heart and possibly treat cardiovascular diseases.

EVs have emerged as a new class of nanocarriers and have been used to deliver RNA, DNA, protein and others. A list of the ongoing clinical studies that use EVs to treat different kinds of disease is provided in *Table 1*.

Currently, there is no ongoing clinical study on treatment of obesity-associated cardiovascular disease with EVs. Nevertheless, an interesting example on how EV could be used in the prevention of cardiovascular disease is the delivery of low-density lipoprotein receptors (LDLR) via EVs to homozygous familial hypercholesterolemia (FH) patients (*Table 1*). FH is an autosomal dominant genetic disease characterized by severely elevated plasma LDL cholesterol and premature coronary heart disease. Most FH patients (about 95% of cases) are attributed to functional loss mutation of the *LDLR* gene (23). Although a few drugs such as statins, ezetimibe, PCSK9 inhibitors and lomitapide are available for treatment at high doses, some patients

Table 1 Ongoing clinical trials using EV as therapeutic agents

Name	EV source	Drug	Disease	Phase	ClinicalTrials.gov identifier
ExoFlo™	Bone marrow MSC-derived EV	No	SARS-CoV-2 ARDS SARS-CoV-2 pneumonia	2	NCT04493242
AGLE-102	Normal donor MSC-derived EVs	No	Epidermolysis bullosa	1/2A	NCT04173650
Exosomes	Adipose-derived SC exosomes	No	Periodontitis	Early 1	NCT04270006
miR-124-loaded exosomes	MSCs-derived exosome loaded with miR-124	miR-24	Cerebrovascular disorders	1/2	NCT03384433
Nebulized MSCs-Exo	MSCs-derived exosomes	No	SARS-CoV-2 pneumonia	1	NCT04276987
EXO-CD24	Exosomes overexpressing CD24	CD24	COVID-19	2	NCT04969172
CovenD24	Exosomes overexpressing CD24	CD24	Severe COVID-19	2	NCT04902183
ARDOXSO™	MSCs-derived exosomes	No	SARS-CoV-2 ARDS SARS-CoV-2 pneumonia	1/2	NCT04798716
Autologous exosomes	Plasma-derived exosomes	No	Ulcer	Early 1	NCT02565264
PRPex	PRP with exosomes	No	Chronic low back pain Degenerative disc disease	1	NCT04849429
CelliStem®OA-sEV	MSC-derived exosomes	No	Osteoarthritis, knee	1	NCT05060107
Exosome of MSC	MSC-derived exosomes	No	Multiple organ failure	–	NCT04356300
haMPC-Exos	Human adipose-derived MS progenitor cell exosomes	No	Carbapenem-resistant gram-negative bacilli-induced pulmonary infection	1/2	NCT04544215
Exo-cur	Curcumin-enriched plant exosomes	Curcumin	Colon cancer	1	NCT01294072
Exosome-based LDLR mRNA nanoplatform	*LDLR* mRNA-enriched exosomes	*LDLR* mRNA	FH	1	NCT05043181
COVID-19 STCs-Exo therapy	COVID-19 specific T cell derived exosomes	No	Coronavirus infection Pneumonia	1	NCT04389385
CB-MSC	Umbilical cord-blood derived MSC EV	No	Diabetes mellitus type 1	2/3	NCT02138331
UMSC-Exo	Umbilical MSC-derived exosomes	No	Dry eye	1	NCT04213248
iExosomes	Mesenchymal stromal cells-derived exosomes with *KRAS G12D* siRNA	KRAS G12D siRNA	Drug-resistant pancreatic adenocarcinoma	1	NCT03608631
MSC-Exo	MSC-derived exosomes	No	Macular holes	Early 1	NCT03437759
MSCs-Exos	Adipose MSCs-derived exosomes	No	Alzheimer disease	1/2	NCT04388982
ExoSTING	EV loaded with cyclic dinucleotide STING agonist	Cyclic dinucleotide	Advanced solid tumor	1/2	NCT04592484

Accessed https://clinicaltrials.gov/ on 2nd December 2021. EV, extracellular vesicle; MSC, mesenchymal stem cell; ARDS, associated acute respiratory distress syndrome; COVID-19, coronavirus disease caused by the SARS-CoV-2 virus; PRP, platelet rich plasma; LDLR, low-density lipoprotein receptors; FH, familial hypercholesterolemia; STING, stimulator of interferon genes.

do not respond and need lipoprotein apheresis or liver transplantation (23). Thus, delivering LDLR via EV could be a safe form of gene therapy and it is under evaluation in this clinical trial.

Despite the fact many studies are emerging considering EV, mainly exosomes, as therapeutics and diagnostic tools, there are many obstacles in the EV field that need to be overcome to achieve success in future strategies of treatment/diagnosis.

EVs are heterogeneous in terms of size, cargo content and cell origin, but current techniques of isolation cannot obtain pure populations of EV. Thus, more tissue-specific biomarkers of EV are necessary to perhaps distinguish EV source and obtain purer preparations.

Questions concerning the source of EV in circulation, the regulation of exosome secretion, targeting of exosomes to specific destinations, and cell specific uptake of EV by recipient cells remains to be answered and should be addressed in the near future. Currently, we have been using acute inhibition of n-SMase2 to inhibit EV secretion, however n-SMase2, an enzyme that generates the bioactive lipid ceramide through the hydrolysis of the membrane lipid sphingomyelin, is not a specific protein for EV generation and is involved in many other cellular processes including cell growth, inflammatory signaling and apoptosis ceramide production (24). Future studies using genetic techniques to create tissue-specific knockout to inhibit release of EV in specific tissues of interest are needed; these animal models should answer not only questions of EV origin in circulation, but perhaps of the role of EV in normal function of these tissues.

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