Exosomes: Cell Garbage Can, Therapeutic Carrier, or Trojan Horse?

Running title: Thébaud et al.; Therapeutic potential of exosomes

Bernard Thébaud, MD, PhD\textsuperscript{1,2}; Duncan J. Stewart, MD\textsuperscript{1}

\textsuperscript{1}Ottawa Hospital Research Institute, Regenerative Medicine Program, Sprott Stem Cell Research Center, University of Ottawa; \textsuperscript{2}Dept of Pediatrics, Children’s Hospital of Eastern Ontario Research Institute, Ottawa, Ontario, Canada

Address for Correspondence:
Bernard Thébaud, MD, PhD
Ottawa Hospital Research Institute
The Ottawa Hospital
501 Smyth Road
Ottawa, ON, K1H 8L6, Canada
Tel: 613-737-8899 ext 73905
Fax: 613-739-6294
E-mail: bthebaud@ohri.ca

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In multicellular organisms, cells communicate with each other via extracellular molecules such as nucleotides, lipids, short peptides, or proteins. These molecules are released in the extracellular space to bind to receptors on other cells, thus modifying intracellular signaling in the recipient cells. In addition to these single molecules, cells also release in their extracellular environment a series of complex structures called membrane vesicles, which contain numerous proteins, lipids, and even nucleic acids\(^1\). Although known for several decades to exist, membrane vesicles have long been thought of as mere cell debris. Over the past few years, however, accumulated evidence has shown that vesicles can affect the cells they encounter by acting as signaling vehicles containing a cell-specific cargo of proteins, lipids, and genetic material that are moved to other cells where they alter function and physiology. The topic of extracellular vesicle-mediated cell-cell communication has now blossomed into a full-fledged field of research.

The three main populations of extracellular vesicles are commonly classified based on their intracellular origin: apoptotic bodies (50 nm–5 \(\mu\)m in size, include DNA, RNA, and histones, and display surface markers, causing them to be rapidly cleared by macrophages), microparticles (50–1000 nm in size, also referred to as microvesicles, ectosomes, shedding vesicles, microparticles, plasma membrane-derived vesicles, or exovesicles), and exosomes.

Exosomes are 40–100 nm in size and represent a specific subtype of secreted membrane vesicles formed through the fusion of multivesicular endosomes with the plasma membrane\(^2\). The endosomal system controls the uptake and processing of various types of macromolecules from the extracellular milieu and the plasma membrane into the cell. It consists of different interconnected vesicular organelles, which include primary endocytic vesicles, early endosomes, recycling endosomes, late endosomes, and lysosomes. Late endosomes bud off parts of their
membrane into their lumen to form intraluminal vesicles. These multivesicular bodies were originally thought to fuse with lysosomes to reduce their intraluminal cargo. However, it became clear that multivesicular bodies can also fuse with the plasma membrane to release their intraluminal vesicles as exosomes into the extracellular space.

Although originally described in 1983 by Harding et al., exosomes received little attention until the discovery of their ability to modulate the immune system, and their potential to transfer genetic information due to the presence of mRNA and miRNA. Since then, the multiple functions of exosomes in immune modulation, tumorigenesis, and propagation of neurodegenerative disease have become evident. This has sparked interest in exosomes as biomarkers and therapeutic targets.

In this issue of *Circulation*, Lee and colleagues explore the therapeutic potential of mesenchymal stromal cell (MSC)-derived exosomes for the prevention of hypoxia-induced pulmonary hypertension in mice. The rationale is based on previous studies where engraftment and direct tissue repair was not necessarily required for MSC therapeutic function, but that paracrine mechanisms accounted for much of the therapeutic benefit of MSCs. These studies revealed that a single injection of cell-free conditioned media derived from MSC exerted long-lasting therapeutic effects.

In the current study, the authors fractionated mouse and human cord MSC-conditioned media and show that the biologically-active component includes exosomes. MSC-derived exosomes (MEX) had pleitropic effects and attenuated lung macrophage influx, as well as decreased pro-inflammatory cytokine levels in the bronchoalveolar lavage. This was associated with prevention of pulmonary vascular remodeling and hypoxic-induced pulmonary hypertension in the mice studied. Fibroblast-derived exosomes and MEX-depleted media had no
effect.

More interestingly, and in accordance with previous exosome literature\textsuperscript{5}, MEX suppressed the hypoxic activation of STAT3 and upregulation of the miR-17 superfamily of microRNA clusters, whereas it increased lung levels of miR-204, a microRNA recently shown to be decreased in human pulmonary hypertension.

Thus, Lee and colleagues provide the first demonstration of the therapeutic potential of MSC-derived exosomes for pulmonary hypertension\textsuperscript{9}. Their findings open exciting avenues given the relevance for other multi-factorial diseases. Indeed, the discovery of the paracrine effect of stem cells has led to the identification of a variety of bioactive molecules secreted by MSCs\textsuperscript{12}. Each of these may contribute to the therapeutic benefit, but it is more likely the combination of these molecules that provide the compounding pleiotropic effects attributed to MSCs. However, the opportunity to capitalize on “cell-based therapies without the cell” brings with it an enormous challenge, such as the need to identify all of the bioactive molecules and determine which combination is most effective. Exosomes are ideal therapeutic agents because their complex cargo of proteins and genetic material has the diversity and biochemical potential to participate in multiple biochemical and cellular processes, a key attribute in the treatment of complex diseases\textsuperscript{13, 14}.

One important challenge in studying exosomes is the lack of robust, reproducible isolation in order to obtain highly pure and well-characterized vesicular populations. Thus far, three different methods have been proposed to isolate and characterize exosomes: (i) ultracentrifugation\textsuperscript{15}, (ii) ultrafiltration\textsuperscript{16}, and (iii) immunoprecipitation technologies using antibody-loaded magnetic cell beads\textsuperscript{17}. Only electron microscopy allows detailed visualization of exosomes making it difficult to assess their function \textit{in vivo}\textsuperscript{18}. Exosomes float on a sucrose
density gradient at heights of 1.13–1.19 g/mL, and contain specific proteins that are incorporated during the formation process in multivesicular bodies, such as Alix, TSG101, and tetraspanins (CD9, CD63), which are frequently used as identification markers. However, there is a current lack of consensus as to the optimal methods for the isolation of pure vesicular preparations. This situation is reminiscent of the MSC field before the description of the minimal criteria for defining multipotent mesenchymal stromal cells, although knowledge about MSCs is still evolving\(^9\).

Controversy also exists between the beneficial and harmful effects of exosomes. In particular, their ability to facilitate viral, tumor or cell damage spreading. On a per particle basis, exosome-associated particles are 10-fold more infectious than cell-free virus particles\(^20\). The ‘‘Trojan exosome hypothesis’’ proposes that enveloped retroviruses such as HIV utilize the host cell’s endosomal system, including the exosome pathway, to move between antigen presenting cells while searching for the best target cells\(^21\). Exosomes have also been implicated in aiding tumor survival and metastasis by carrying cancer associated proteins that induce the enhanced cell growth, angiogenesis, and transformation of other cells, while evading the immune response by being encapsulated in the circulating exosomal delivery system\(^7\). Finally, exosomes may also mediate the spread of cell damage or alter the microenvironment in various metabolic and nervous system disorders. The ‘‘Trojan Exosome’’ model of Alzheimer’s disease proposes that the initial insult of Alzheimer’s may spread to surrounding cells via exosomes laden with amyloid beta peptide and other proteins and RNA associated with precipitating Alzheimer’s pathogenesis\(^8\).

However, the potential of exosomes is already being exploited in the clinic (\textit{http://clinicaltrials.gov}) as therapeutic carrier to treat patients with lung cancer and as biomarker
to monitor the therapeutic responses in breast cancer patients.

Considered for a long time as little more than cellular garbage cans acting to discard unwanted molecular components, exosomes have now elevated to the status of potentially powerful biomarkers and possible vehicles for the delivery of therapeutic molecules. Rapid progress in parallel disciplines, including bioengineering\(^2\),\(^3\) and systems biology (ExoCarta, a freely accessible web-based compendium of proteins and RNAs found in exosomes http://exocarta.ludwig.edu.au/) will further advance our understanding of exosomes, and facilitate isolation, characterization, quality control, and large-scale manufacturing for diagnostic and therapeutic strategies for patients with life-threatening and debilitating diseases. In this issue, Lee and colleagues contribute to this growing knowledge base by extending the therapeutic potential of these vesicles to patients with pulmonary hypertension\(^9\).

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