Explicating Exosomes: Reclassifying the Rising Stars of Intercellular Communication

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There is growing interest surrounding the diagnostic and therapeutic potential of exosomes, but a definitive description of these extracellular vesicles remains elusive. In this issue, Jeppesen et al. characterize exosomes following a strict isolation protocol and in so doing challenge several of the accepted properties of these agents of intercellular communication.

The secretion of membranous extracellular vesicles (EVs) by cells is a phenomenon well conserved across biological domains. Although the physiological function of these EVs has been recognized in a few specific contexts, such as neurotransmission or endocrine signaling, the broader process of vesicle secretion had been largely dismissed as a waste-disposal mechanism. Nevertheless, with mounting evidence of vesicles trafficking biologically active cargos that vary in response to their microenvironment, EVs have come to be accepted as ubiquitous mediators of intercellular communication among all cell types. EVs transfer non-mediators of intercellular communication have come to be accepted as ubiquitous response to their microenvironment, EVs mounting evidence of vesicles trafficking disposal mechanism. Nevertheless, with mounting evidence of vesicles trafficking biologically active cargos that vary in response to their microenvironment, EVs have come to be accepted as ubiquitous mediators of intercellular communication among all cell types. EVs transfer non-mediators of intercellular communication have come to be accepted as ubiquitous response to their microenvironment, EVs mounting evidence of vesicles trafficking disposal mechanism. Nevertheless, with mounting evidence of vesicles trafficking biologically active cargos that vary in response to their microenvironment, EVs have come to be accepted as ubiquitous mediators of intercellular communication among all cell types. EVs transfer non-mediators of intercellular communication have come to be accepted as ubiquitous response to their microenvironment, EVs mounting evidence of vesicles trafficking disposal mechanism.

EVs more generally encompass a variety of vesicle types that differ in their route of biogenesis, size, and composition, with exosomes typically being defined as small vesicles, 40–150 nm in size, arising from a multivesicular endosomal (MVE) pathway. A number of different techniques are routinely used to isolate exosomes from biological fluids, including differential centrifugation, density-gradient centrifugation, and ultrafiltration. Each technique comes with its own advantages and disadvantages, and critically, they yield preparations of small EVs that differ in purity and heterogeneity. Thus, characterization of the composition, cargo, and functionality of these populations are likely to reflect a diversity of vesicular and non-vesicular components rather than exosomes specifically. This heterogeneity is one key factor driving controversies surrounding the properties of exosomes, such as whether they are capable of cell-specific uptake and if their nucleic acid cargos are functional upon delivery. A more specific understanding of the biological role and therapeutic potential of exosomes and other EVs necessitates a more discriminating isolation and classification, which is what Jeppesen et al. (2019) now report.

The authors first remove cell debris and large EVs by centrifugation before employing a two-step isolation procedure on the resultant crude small EV (sEV) preparation: high-resolution density-gradient fractionation to separate sEVs from non-vesicular material followed by separation of exosomes from other (non-exosomal) sEVs by direct immunoaffinity capture (DIC). The end result of this process is a population of vesicles of the appropriate density and bearing the tetraspan exosomal markers CD9, CD63, and CD81. The composition of these classical exosomes differs from that of orthodox exosome preparations in a number of ways. First, classical exosomes are found to be absent luminal proteins commonly though ubiquitous to exosomes, including enzymes such as glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and enolase and the chaperone heat shock protein 90 (HSP90). Cytoskeletal proteins are also not found by the authors inside classical exosomes, which, combined with the absence of metabolic enzymes, speaks against random encapsulation of the parent cell’s cytosol during exosome biogenesis and instead implies controlled cargo packaging mechanisms. Additionally, membrane-bound annexins A1 and A2—often considered characteristic of exosomes—are instead to be markers of non-exosomal sEVs (Figure 1).

This work also addresses some of the contentious issues surrounding exosomal miRNA functionality, with classical exosomes found to be absent the Argonaute (Ago) proteins 1–4, components of the RNA-induced silencing complex, and all other miRNA-associated enzymes (e.g., Dicer, Drosha). Thus, in contrast to previous findings (Melo et al., 2014), classical exosomes lack the necessary componentry to facilitate cell-independent miRNA biogenesis. Indeed, with the exception of traces of Ago1–4 being detected in
non-exosomal sEVs from a single cell line, components of the miRNA machinery are not observed in any sEVs. Patterns of miRNA abundance differ between cellular and extracellular sources, with the latter showing further disparity between non-vesicular and sEV fractions. Furthermore, none of the frequently cited extracellular RNA-binding proteins are found to be associated with EV fractions isolated by DIC, which suggests that classical exosomes may not be a significant source of extracellular RNA.

Perhaps most significantly, the authors demonstrate that extracellular double-stranded DNA is not associated with classical exosomes, or indeed any sEVs, but is instead co-purified with these EVs during standard isolation techniques. An EV-independent extracellular secretion mechanism is instead proposed, wherein DNA and histones are actively released alongside sEVs in an autophagy- and MVE-dependent pathway. Given the growing interest in extracellular DNA as a disease marker in liquid biopsies, a re-assessment of what is actually being measured may be due. Indeed, future studies should ideally demonstrate that components or functions of interest are indeed characteristic of a specific EV versus non-vesicular population. However, such precision may come at a cost—i.e., the methodology used in this study is more costly and less efficient than traditional methods with respect to time and yield. Ultimately, there is a need for greater standardization of isolation and purification techniques, or even a revision of the current classification and nomenclature. At a deeper level, this work highlights the need for a deeper understanding of the mechanisms underlying the biogenesis and loading of EVs, with issues such as the lipid composition of the exosomes and the mechanisms of miRNA packaging needing further study.

Jeppesen et al. (2019) show that bona fide exosomes have a substantially more restricted repertoire of biomolecular constituents than has been generally accepted. While the criterion for classifying exosomes is intrinsically arbitrary, precision in scientific nomenclature is vital for ensuring consistency among experimental observations.

Correctly attributing functionality to the appropriate extracellular entity will be imperative for their successful use as biomarkers or therapeutics.

**DECLARATION OF INTERESTS**

Stefano Pluchino owns >5% of CITC Ltd.; Jayden A Smith is an employee at CITC Ltd.

**REFERENCES**


