Published in final edited form as:

Nat Cell Biol. 2018 March ; 20(3): 228–230. doi:10.1038/s41556-018-0049-8.

Size matters in nanoscale communication

Andries Zijlstra¹Dolores Di Vizio²

¹Department of Pathology, Microbiology and Immunology, Vanderbilt University Medical Center, Nashville, TN, USA

²Department of Surgery, Department of Biomedical Sciences, Department of Pathology and Laboratory Medicine, Cedars-Sinai Medical Center, Los Angeles, CA, 90048, United States

Abstract

Exosomes are heterogeneous, nanoscale vesicles that mediate cellular communication. A study now leverages a size separation strategy to identify sub-classes of nanoparticles. Besides revealing a subtype without an encapsulating membrane, biochemical analyses show variation in vesicle cargo, suggesting that size is not the only driver of heterogeneity.

Extracellular vesicles, of which exosomes represent one nanoscale subpopulation¹, have been recognized as *bona fide* promoters of cancer progression because they function as mediators of communication between the cell of origin and adjacent cells as well as distant tissues^{1,2}. The underpinnings of this mechanism of communication and the nature of what is being communicated have been under intense investigation over the past five years. An active and specialized role for cancer-derived extracellular vesicles in tumor progression has been confirmed, which includes communication with and transfer of active biomolecules to the tumor microenvironment³. In addition, a functional role for extracellular vesicle cargo sorting in disease progression has been demonstrated and the mechanism revealed⁴. The inhibition of exosome secretion or biogenesis also leads to defective tumour cell migration⁵, suggesting that the tumor-promoting function of extracellular vesicles can be therapeutically targeted^{6,3}.

In this issue of⁷ Nature Cell Biology, Zhang et. al. implemented asymmetric flow-field fractionation (AF4) of nanoscale particles released by cells to classify these components of the cellular communication milieu according to size. In doing so, this work not only confirmed the presence of at least two previously reported exosome subclasses⁸, named small and large exosomes, but also identified a previously unappreciated nanoparticle, termed exomere, which is a discrete and abnormally small nanoparticle of about 35 nm in diameter that exhibits a distinct protein, lipid, RNA and DNA profile compared to exosomes.

Zhang et. al. performed an in-depth molecular analysis to compare the cargo of the exomeres with two different exosome populations (large exosomes, Exo-L, and small exosomes, Exo-S), purified from the same cell type. Their findings indicate that the two exosome subpopulations have distinct biophysical and molecular properties, which differ from those

of exomeres. More specifically, the three nanosized particles displayed diverse proteomic profiles, N-Glycosylation patterns, lipidic, RNA and DNA profiles, suggesting that they might originate from different biogenesis mechanisms. Moreover, once injected into the bloodstream of animals, the three particles also exhibited different biodistribution. The authors therefore propose that exosomes and exomeres are likely to play different intercellular functions in inter-cellular communication.

Possibly the most compelling question in the extracellular vesicle field is the mechanism by which heterogeneity of nanoscale particles is accomplished. Size and cargo heterogeneity among extracellular vesicles is now commonly appreciated, with substantial evidence in the literature^{8–10}. In addition, micro-sized vesicles, such as exophers, migrasomes and large oncosomes^{11–13}, have been discovered by several laboratories (Figure 1). It is generally presumed that this diversity is the result of distinct biogenesis mechanisms¹⁴. However, only the biogenesis of exosomes has been revealed in some detail to date¹⁵.

Zhang et al. further found that exomeres were enriched in proteins involved in metabolism, especially glycolysis and mTORC1 metabolic pathways. These results suggest that exomere biogenesis involves subcellular organelles or activity associated with the cell's metabolism, such as mitochondrial function. Similarly, they speculate that the receiving cells are likely to interpret this exomere-delivered signal in the form of a metabolic response. The authors demonstrated that exomeres were also enriched in proteins associated with the endoplasmic reticulum, mitochondria and microtubules, proposing a potential role for these proteins in exomere biogenesis and secretion. However, these classes of proteins have been previously attributed to exosomes. Another interesting observation was that even though exomeres did not have a lipid bilayer, they were enriched in certain types of lipids, which differ from those found in Exo-S and Exo-L. In addition, DNA packaging in exomeres and exosomes varied by tumor-type, whereas RNA was packaged in Exo-S and Exo-L independently of the tumor cells they originated from.

The analysis of nanoparticle cargo revealed differences not only between each size subclass, but also within each class. Previously, expression of CD9, CD63, CD81, Tsg101 and Alix1 have been considered as specific markers of exosomes. However, in this work they were distributed very differently between Exo-L and Exo-S, suggesting that more than one type of exosome populations exists. These data are in line with a previous study reporting that extracellular vesicle populations are heterogeneous⁸. The detailed, in-depth analysis of the particle composition performed by the authors demonstrated that each particle subclass had a notable enrichment of specific cargo, possibly indicating different functions and properties. Compared to Exo-S and Exo-L, exomeres were also enriched in enzymes, suggesting potential roles in influencing the metabolic program in target organ cells, as well as in proteins associated with coagulation (for instance, Factors VIII and X) and hypoxia. Conversely, Exo-S predominantly contained proteins associated with endosomes, multi-vesicular bodies, vacuoles, and phagocytic vesicles, whereas Exo-L were specifically enriched in proteins that make up the plasma membrane, cell-cell contacts or junctions, the late-endosome and trans Golgi network. These findings suggest that Exo-S are most likely *bona fide*, canonical exosomes, for example derived from intraluminal vesicles of endosomal compartments. By contrast, Exo-L may represent non-canonical exosomes or extracellular

vesicles of different sub-cellular origin, for instance derived from plasma membrane budding.

Although enrichment of certain cargos within a subclass indicates commonality in biogenesis and function, the authors also revealed substantial intra-class heterogeneity evident in the cargo variability between cell lines. By comparing several cancer cell lines, including breast cancer, pancreatic cancer and melanoma in parallel, Zhang et al. confirmed that even though each cell line contained the three nanoparticle subclasses, the composition of their cargo was very much dependent on the cell line from which they are derived. Although this result is not unexpected, it additionally corroborates that heterogeneity among nanoparticle populations is driven not only by size but also by cargo composition. Considering that the cargo will dictate, in large part, the response of the recipient cell to the particle, the mechanisms that control cargo composition in different types of particles will be an interesting matter to focus on in future studies.

One of the most important implications of this study is that it shows that extracellular vesicle heterogeneity is not only defined by variation in size, but also by variations in cargo between and within each size class. Although the possibility that exomeres are simply aggregates of molecules rather than extracellular vesicles was not ruled out, the finding that exomeres contain a complex array of macromolecules corroborates the hypothesis that exomeres are indeed a unique class of nanoparticle with, as of yet, unknown function. If true, then exomeres add another dimension to the heterogeneity of the nanoparticle communication milieu, which, if better understood, might allow improved insights into cellular communication mechanisms and also provide a previously overlooked biomarker. The discovery of exomeres and stratification of exosomes using AF4 emphasizes the effectiveness of this technique and the potential to reveal additional populations of particles. One limitation of the study is the lack of functional experiments. However, the difference in cargo, and especially in N-glycosylation, which has been rarely investigated in extracellular vesicles, highly suggests functional differences that can be investigated in the future. Collectively, this comprehensive investigation will certainly open new avenues for translational studies of extracellular vesicles and particles in diagnostic, prognostic, and therapeutic applications.

Reference list

1. Lötvald J et al. Minimal experimental requirements for definition of extracellular vesicles and their functions: a position statement from the International Society for Extracellular Vesicles. *J Extracell Vesicles* 3, 26913 (2014). [PubMed: 25536934]
2. Peinado H et al. Melanoma exosomes educate bone marrow progenitor cells toward a pro-metastatic phenotype through MET. *Nat. Med.* 18, 883–891 (2012). [PubMed: 22635005]
3. van der Vos KE et al. Directly visualized glioblastoma-derived extracellular vesicles transfer RNA to microglia/macrophages in the brain. *Neuro. Oncol.* 18, 58–69 (2016). [PubMed: 26433199]
4. Clancy JW et al. Regulated delivery of molecular cargo to invasive tumour-derived microvesicles. *Nat. Commun.* 6, 6919 (2015). [PubMed: 25897521]
5. Sung BH, Ketova T, Hoshino D, Zijlstra A & Weaver AM Directional cell movement through tissues is controlled by exosome secretion. *Nat. Commun.* 6, 7164 (2015). [PubMed: 25968605]
6. Kamekar S et al. Exosomes facilitate therapeutic targeting of oncogenic KRAS in pancreatic cancer. *Nature* 546, 498–503 (2017). [PubMed: 28607485]

7. Zhang H et al. Identification of distinct nanoparticles and subsets of extracellular vesicles by asymmetric-flow field-flow fractionation. *Nature Cell Biology*. (2018)
8. Kowal J et al. Proteomic comparison defines novel markers to characterize heterogeneous populations of extracellular vesicle subtypes. *Proc. Natl. Acad. Sci. U. S. A.* 113, E968–77 (2016). [PubMed: 26858453]
9. Xu R, Greening DW, Rai A, Ji H & Simpson RJ Highly-purified exosomes and shed microvesicles isolated from the human colon cancer cell line LIM1863 by sequential centrifugal ultrafiltration are biochemically and functionally distinct. *Methods* 87, 11–25 (2015). [PubMed: 25890246]
10. Crescitelli R et al. Distinct RNA profiles in subpopulations of extracellular vesicles: apoptotic bodies, microvesicles and exosomes. *J Extracell Vesicles* 2, (2013).
11. Melentijevic I et al. *C. elegans* neurons jettison protein aggregates and mitochondria under neurotoxic stress. *Nature* 542, 367–371 (2017). [PubMed: 28178240]
12. Ma L et al. Discovery of the migrasome, an organelle mediating release of cytoplasmic contents during cell migration. *Cell Res.* 25, 24–38 (2015). [PubMed: 25342562]
13. Minciacchi VR et al. MYC Mediates Large Oncosome-Induced Fibroblast Reprogramming in Prostate Cancer. *Cancer Res.* 77, 2306–2317 (2017). [PubMed: 28202510]
14. Minciacchi VR, Freeman MR & Di Vizio D Extracellular vesicles in cancer: exosomes, microvesicles and the emerging role of large oncosomes. *Semin. Cell Dev. Biol.* 40, 41–51 (2015). [PubMed: 25721812]
15. Ostrowski M et al. Rab27a and Rab27b control different steps of the exosome secretion pathway. *Nat. Cell Biol.* 12, 19–30; sup pp 1–13 (2010).

Figure 1. Schematic representation of vesicle subpopulations that comprise the cellular communication milieu.

Stratified extracellular vesicles, including the recently identified exomeres, listed according to size ranges. Whereas the biogenesis of exosomes is relatively well-defined, the biogenesis of larger vesicles and exomeres is currently unknown. Extracellular vesicles contain cargo material from the cytoplasm, mitochondria, Golgi, the plasma membrane, endoplasmic reticulum and nucleus. Abbreviations: EV, extracellular vesicle; MVB, multivesicular bodies.