

Title: Mapping the methodological diversity of EV isolation and characterization

Authors: Jan Van Deun(1), Pieter Mestdagh(2), Jo Vandesompele(2), Olivier De Wever(1), An Hendrix(1)

Affiliations: (1)Laboratory of Experimental Cancer Research, Department of Radiation Oncology and Experimental Cancer Research and (2)Center for Medical Genetics, Ghent University Hospital, Ghent, Belgium.

Abstract:

Extracellular vesicles (EVs), most notably exosomes, are a hot topic in medical research. While a small, dedicated part of the scientific community is struggling to determine the defining characteristics of different subtypes of EVs, as well as with finding effective ways to isolate them, their putative functions are featured in an exponentially increasing number of publications. This dangerous hiatus between our limited basic knowledge and the extensive functions that are being attributed to EVs is only highlighted by the fact that studies often do not properly report how the EVs were isolated and characterized. When reported, there is a variety of methods at hand to isolate and characterize EVs, further complicating the matter.

After undertaking an unprecedented in-depth analysis of the scientific literature on EVs published in the last 6 years, comprising over 1200 articles and investigating ~100 variables associated with isolation and characterization methods, we found an extensive diversity among publications. E.g., when looking only at publications reporting on EVs from cell culture supernatant, about 1 in 2 report a different (combination of) isolation methods that was used. About 2 in 3 papers use a different characterization procedure for their isolates. The overall level of characterization is poor, with only 15% of papers providing a combination of protein analysis, individual particle analysis and electron microscopy data, as would be appropriate for a full identification of their isolates.

Based on the most commonly found ambiguities, we propose a checklist specifically for EV researchers that should ensure a more comprehensive and relevant representation and interpretation of EV-related results, with the final aim of substantially enhancing reproducibility in this field.