Optimisation Of Parasitic Extracellular Vesicle Purification for Downstream Analysis To Understand Their Role Within Drug Exposure

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Abstract

Robust protocols for the isolation of parasitic extracellular vesicles (EVs) away from other excretory-secretory (ES) products are necessary for downstream functional studies and applications such as vaccine and diagnostic development. The most widely used purification method of EVs in parasite biology is currently differential centrifugation (DC). Outside of parasite biology, size exclusion chromatography (SEC) has been adopted to purify EVs. However, there is no agreed research community 'gold standard' of EV isolation from parasitic helminths. In this case study, *Fasciola hepatica* from natural populations were cultured in order to collect EVs and evaluate a SEC or DC approach to EV purification focusing on the properties of EV preparations. Transmission electron microscopy and atomic force microscopy demonstrated that EVs prepared by SEC were both smaller in size and diversity than EV populations resolved by DC. Protein concentration and Western blotting indicated that SEC purification realised a high EV purity to free ES protein yield ratio compared to DC approaches. Proteomic analysis highlighted an increased diversity of protein identifications and unique peptide hits in EVs isolated by DC compared to SEC. In contrast, transcription and ribosome GO terms, following gene enrichment analysis, demonstrated significantly less gene enrichment in DC purified EVs compared to SEC purified EVs, while translation was enriched to a greater extent in DC purified EVs compared to SEC purified EVs. This data suggests that DC and SEC purification methods do not isolate equivalent EV population profiles and caution should be taken in the choice of EV purification utilised with functional assays incorporated into the isolation pipeline. Thus, this research highlights SEC methods with functional assays as the methodology of choice for parasite EV studies and application development.

Following EV purification analysis, we further aimed to determine the role of parasite EVs during drug exposure, given that investigations upon the EVs and drugs have been limited to cancer chemotherapy and antibiotic resistance research. Therefore, natural populations of *F. hepatica* were cultured in lethal and sub-lethal doses of triclabendazole, and active metabolites, in order to SEC purify EVs and evaluate their production, morphological characteristics and drug metabolite content. TEM micrographs demonstrated that all drug exposure EV samples had similar morphology despite disruption to the tegument. qNano particle analysis identified that drug exposure samples produced at least five times more EV concentration than drug exposure controls, where drug dose or drug metabolite did not significantly affect EV production. Particle diameter analysis also showed that only under lethal doses of TCBZ-SO did parasites produce smaller EVs. Using mass spectrometry and qNano particle analysis, drug concentrations in EVs were found in all TCBZ and TCBZ-SO drug exposure samples, although little was identified in TCBZ-SO_2 drug exposure samples. Quantification of drug contained within EVs suggests that drug uptake is passive. Interestingly, alternative TCBZ was observed in TCBZ-SO drug exposure samples. This data suggests that EVs may have a metabolic role when parasites are subjected to drug exposure. In this study, it is likely that EVs were utilised to remove drug metabolites from the parasite's microenvironment, to maintain parasite survival. Further research upon the biological role of EVs in parasite environments could provide insight into improving drug control strategies and possibly drug resistance scenarios.