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Technical note

Peptide-mediated 'miniprep' isolation of extracellular vesicles is suitable for high-throughput proteomics

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ABSTRACT

Extracellular vesicles (EVs) are cell-secreted membrane vesicles enclosed by a lipid bilayer derived from endosomes or from the plasma membrane. Since EVs are released into body fluids, and their cargo includes tissue-specific and disease-related molecules, they represent a rich source for disease biomarkers. However, standard ultracentrifugation methods for EV isolation are laborious, time-consuming, and require high inputs. Ghosh and co-workers recently described an isolation method utilizing Heat Shock Protein (HSP)-binding peptide Vn96 to aggregate HSP-decorated EVs, which can be performed at small 'miniprep' scale. Based on microscopic, immunoblot, and RNA sequencing analyses this method compared well with ultracentrifugation-mediated EV isolation, but a detailed proteomic comparison was lacking. Therefore, we compared both methods using label-free proteomics of replicate EV isolations from HT-29 cell-conditioned medium. Despite a 30-fold different scale (ultracentrifugation: 60 ml/Vn96-mediated aggregation: 2 ml) both methods yielded comparable numbers of identified proteins (3115/3085), with similar reproducibility of identification (72.5%/75.5%) and spectral count-based quantification (average CV: 31%/27%). EV fractions obtained with either method contained established EV markers and proteins linked to vesicle-related gene ontologies. Thus, Vn96 peptide-mediated aggregation is an advantageous, simple and rapid approach for EV isolation from small biological samples, enabling high-throughput analysis in a biomarker discovery setting.

Body fluids represent a rich source of disease biomarkers as they pass or perfuse different tissues and can be easily sampled. However, these fluids tend to have a complex composition and exhibit a large dynamic range of protein levels. This has hampered protein biomarker discovery to date. Yet, virtually all biofluids harbor a potential treasure trove in the form of extracellular membrane vesicles (EVs) emanating from cells that, depending on the circumstances, selectively load the vesicles with some of their contents and secrete them into the surroundings [1,2]. EVs are believed to serve intercellular communication and macromolecular shuttling to nearby and distant cells, affecting diverse processes such as those involved in cancer progression [3]. They offer a stabilizing environment for long-distance journeys of their cargo [4], which consists of proteins, nucleic acids and lipids—some of them telltale components that reflect the (state of the) cellular origin of secretion. Thus, EVs carry an informative sub-proteome that is segregated from dominating constituents precluding analysis of whole biofluids. Hence, EVs could be exploited as stockpiles of indicators for pathologic conditions in the parental tissue, a notion for which an encouraging case in point was published recently [5]. Mechanistically, EVs can arise in two ways: 'exosomes' are endosome-derived vesicles released into the extracellular space from the lumen of multivesicular bodies, whereas 'microvesicles' (also termed 'ectosomes') pinch off directly from the plasma membrane and can reach larger sizes [6,7]. As yet there is no 'gold standard' EV isolation method [8], and most procedures yield mixtures of vesicles (there being no distinguishing physicochemical features enabling separation of exosomes and ectosomes) and varying amounts of contaminating material. To date, the most commonly used method involves fractionation of biological fluid through differential centrifugation followed by one or more ultracentrifugation steps to collect crude

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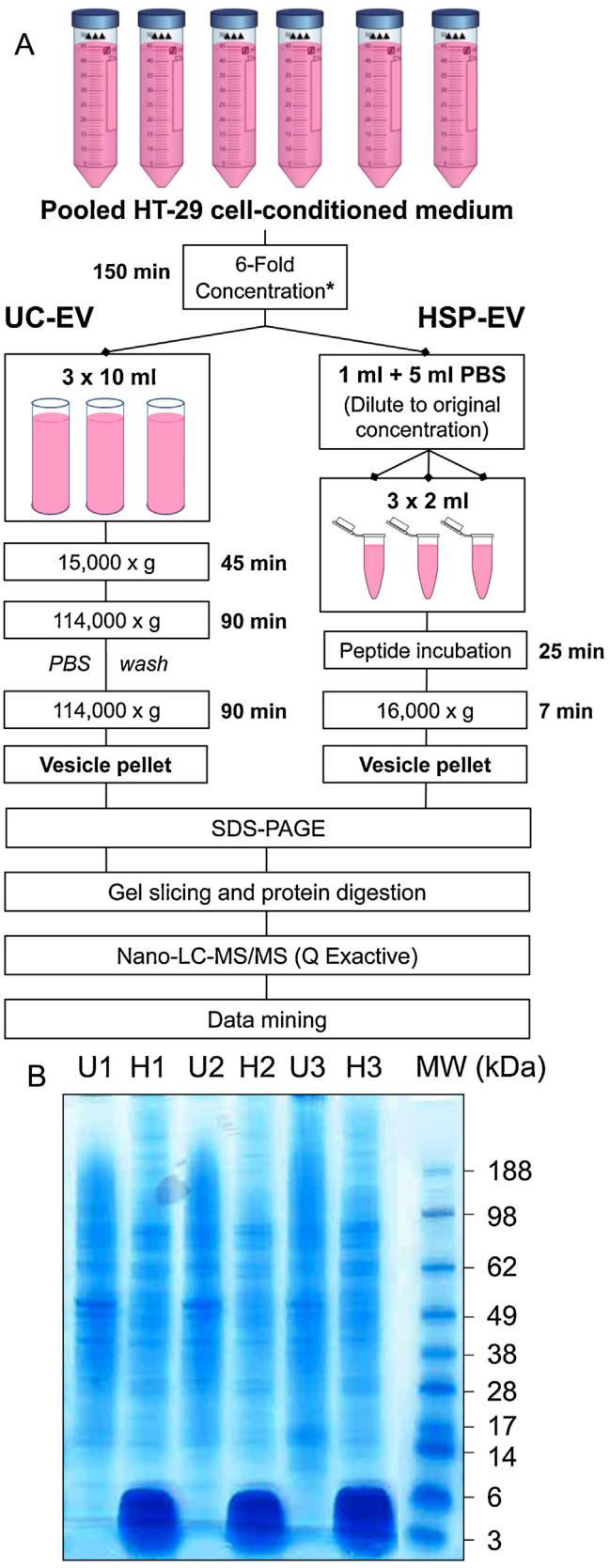

Fig. 1. Proteomic analysis of EVs from HT-29 cell-conditioned medium. (A) General scheme of the workflows for EV isolation by ultracentrifugation (UC-EV, left) and by Vn96-peptide mediated aggregation (HSP-EV, right) as well as common downstream analysis steps. The asterisk in the box denoting "6-fold concentration" indicates that time-consuming concentration was used for both UC-EV and HSP-EV, but only required for UC-EV so as to accomodate samples in ultracentrifugation tubes; it is not needed for the HSP-EV workflow but was included to start with an identical input proteome. (B) Protein gel pattern of all EV fraction replicates (numbered 1–3). U, UC-EV; H, HSP-EV; MW, molecular weight marker.