Revealing unique surface profile of cell-derived vesicles by combining proteome analysis and nanoparticle flow cytometry

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Introduction

The cell-derived vesicles (CDVs) are obtained from virtually any cell by applying MDimune's proprietary extrusion technology. With superior productivity and versatility, this technology has garnered increasing attention as a drug delivery vehicle. We previously described the similarity and differences between CDVs and exosomes. Particularly, among well-known exosome markers, CD9 and CD81 are less represented in CDVs compared to exosomes while CD63 is more prominent in CDVs. In an early survey of CDV markers at the single-particle level, we also found that three tetraspanin markers are differentially expressed between CDVs and exosomes. Therefore, a more systematic survey of marker expression profiles was necessary to better understand CDVs and their therapeutic potentials.

Overview of Study Workflow

A total of three batches of parental cells, CDVs, and exosomes obtained from the parental cells were independently prepared for the study. The triplicate batches were used to enhance the reliability and validity of data analysis. Then, comparative analyses were performed between CDVs, cells, and exosomes.

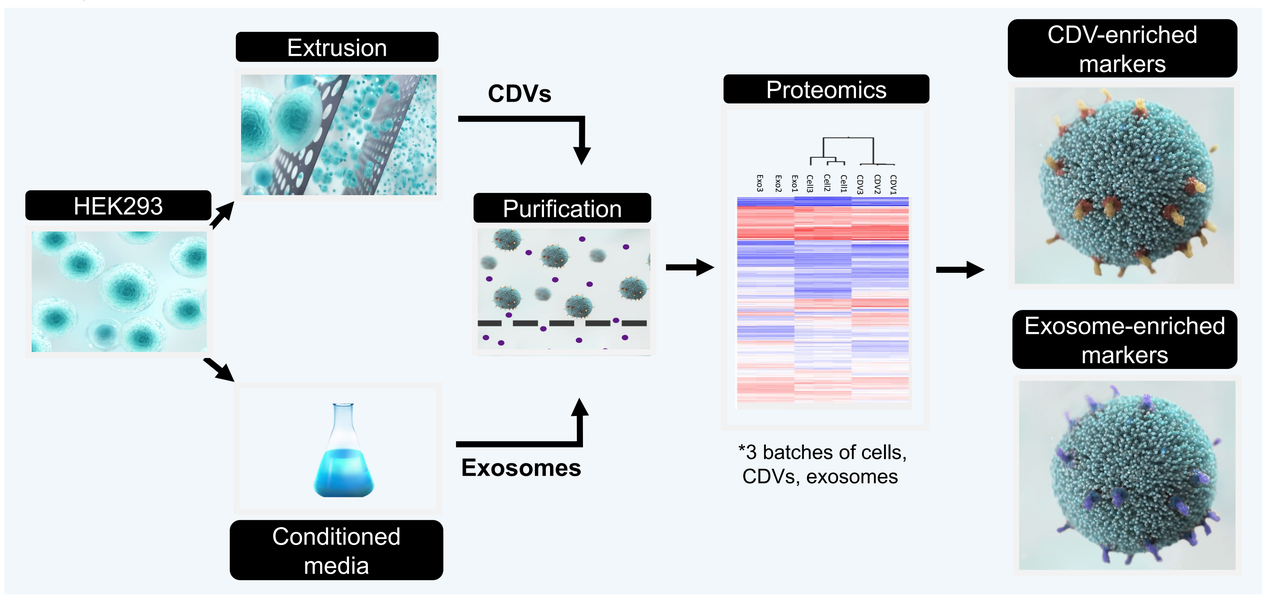
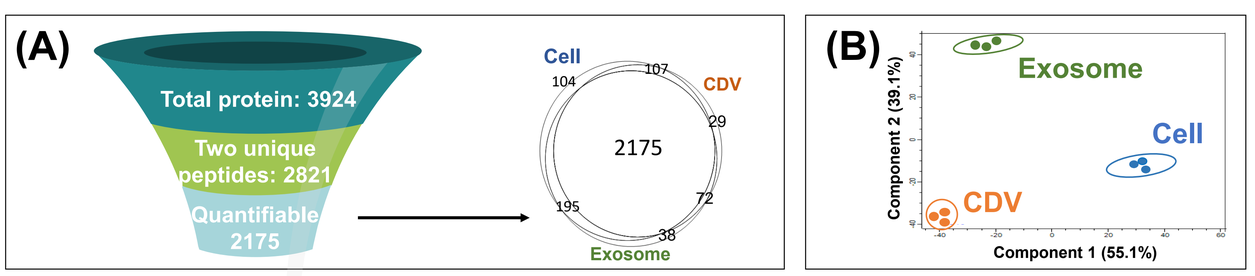


Figure 1. Schematic diagram shows the workflow of the study. HEK-CDVs and exosomes were produced from Lonza's HEK293. Both CDVs and exosomes were subjected to the same purification methods and analyzed for the proteome and unique protein marker expression.

Proteome Analysis

The number of proteins identified from mass-spectrometry were further selected based on the number of hits (at least two peptides per protein). The differentially expressed proteins were identified using the criteria of fold change >2, and *p*-value <0.05 relative to cells.



(C)

Proteome analysis	CDVs	Exosomes
Number of genes	582	519
Total protein abundance (×10^9)	68	69

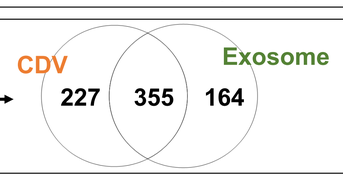


Figure 2. Proteome analysis of HEK-CDVs, exosomes, and their parental cells. (A) Hierarchical pyramid shows the proteome datasets filtering process for the identification of protein markers for all sample groups. The Venn diagram displays the 2175 proteins shared by all the samples. (B) Principal component analysis (PCA) plot analysis revealed that these three groups segregated from each other distinctively, also suggesting consistency of CDV production. (C) Differentially expressed proteins for CDVs and exosomes were further analyzed to select CDV-enriched markers.

Selection of CDV-enriched Membrane Proteins

The unique CDV markers were first selected from the top 20 membrane proteins (by abundance) of the CDV proteome datasets. Among the selected proteins, only the transmembrane proteins with fold change >5 relative to cells were identified as CDV-enriched protein markers. These CDV-enriched markers all showed higher expression than exosomes.

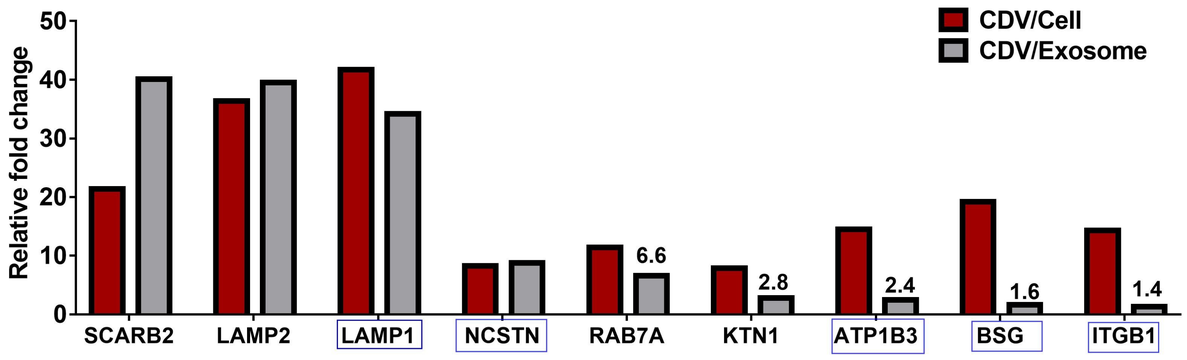


Figure 3. Abundant protein markers highly enriched in CDVs. The expression level in CDVs was compared to cells and exosomes. SCARB2, LAMP1, and LAMP2 showed the highest expression in CDVs compared to cells and exosomes. Others selected CDV markers also had high protein abundances in CDVs relative to cells and exosomes. Protein marker in the blue box were selected for further surface marker analyses while considering the antibody availability.

* **SCARB2**: lysosome membrane protein 2; **LAMP1**: lysosome-associated membrane glycoprotein 1; **LAMP2**: lysosome-associated membrane glycoprotein 2; **NCSTN**: nicastrin; **RAB7A**: ras-related protein Rab-7a; **KTN1**: kinectin; **ATP1B3**: sodium/potassium transporting ATPase subunit beta-3; **BSG**: basigin; **ITGB1**: integrin beta-1.

CDV Surface Marker Analyses

The selected membrane protein markers were analyzed by western blotting and nanoparticle flow cytometry to verify the unique CDV-specific membrane proteins identified from proteome analysis. Exosome-enriched proteins such as tetraspanin markers and prostaglandin F2 receptor inhibitor (PTGFRN) were also compared.

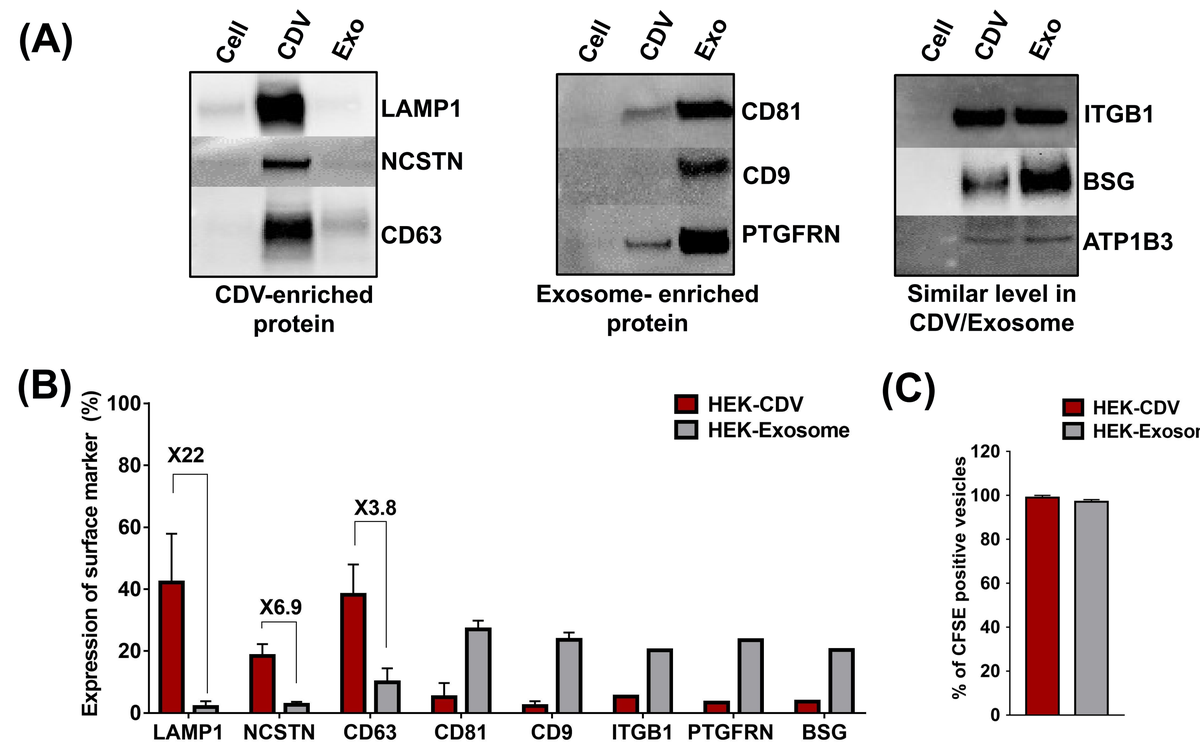


Figure 4. (A) Western blotting analyses confirmed that LAMP1, CD63, and NCSTN were enriched protein markers in CDVs. CD81, CD9, BSG, and PTGFRN were abundant in exosomes. (B) The nanoparticle flow cytometry results were coherent with the western blotting analyses. In contrast, western blotting and nanoparticle flow cytometry results for BSG and ITGB1 did not support proteomics findings. (C) CFSE staining revealed that more than 90% of the CDVs are intact lipid vesicles that retain the membrane integrity.

Conclusion & Future Prospects

- We have identified 3 prominent CDV markers and confirmed that CDVs are intact lipid vesicles that retain the membrane integrity.
- These findings reveal the unique mechanism of CDV biogenesis while assuring the therapeutic potential of CDVs in drug delivery.
- This study will facilitate the development of more sophisticated CDV engineering that enables targeted drug delivery.