MDimune

Identification of anchor proteins for the BioDrone^{TM} platform

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Abstract

BioDrone^{TM}, developed at MDimune, is a state-of-the-art drug delivery system based on cell-derived vesicles (CDVs) produced by serial extrusion from diverse cell sources. CDVs exhibit physicochemical similarity to extracellular vesicles (EVs) but a tremendous advantage in production scalability over EVs. Furthermore, with genetic engineering of mother cells, CDVs can potentially acquire additional biological features such as targeting and cargo loading capabilities. In this study, we identified stable anchor proteins exclusively for HEK-CDV. First, we analyzed the HEK-CDV proteome and selected a set of CDV-specific membrane proteins highly abundant in the CDVs over cells or EVs. Then, these anchor candidates were overexpressed with a tag and GFP in the HEK293 cell. Antibiotic selection and cell sorting established a pool of stable HEK293 cells. After CDV production, the anchor fusion proteins were quantified via GFP ELISA, and their distribution in a single-particle resolution was examined by nanoflow cytometry. Four HEK-CDV anchors among candidates showed a stable presence in the CDVs, with 30 to 150 molecules per CDV particle and 42 to 66 % of the GFP (+) population among total particles, depending on the anchor proteins. Moreover, their proper topology was confirmed via protease cleavage assay, while their abundance in the CDVs was also confirmed from a large-scale extrusion. Together, we present a set of HEK-CDV anchors for the versatile engineering of the BioDrone platform. Currently, we are developing various BioDrone therapeutics utilizing these anchors.

Proteome Analysis of HEK-CDV

HEK-CDV proteome analysis revealed several anchor candidate proteins which were exclusively abundant in HEK-CDV compared to cell and exosome.

Figure 1. Identification of anchor candidates from HEK-CDV via proteome analysis. (A) Comparative proteome analyses of CDV vs. cell and exosome. (B) Percentage of diverse subcellular membrane origin in HEK-CDV. (C) A list of proteins for HEK-CDV anchoring candidate.

Generation of Stable Cell Lines Overexpressing Anchor Candidates

Anchor candidates fused with EGFP were transduced to HEK293 cells using lentiviral particles. Antibiotic selection and cell sorting on transduced cells eliminated untransduced cells and generated stable cell lines.

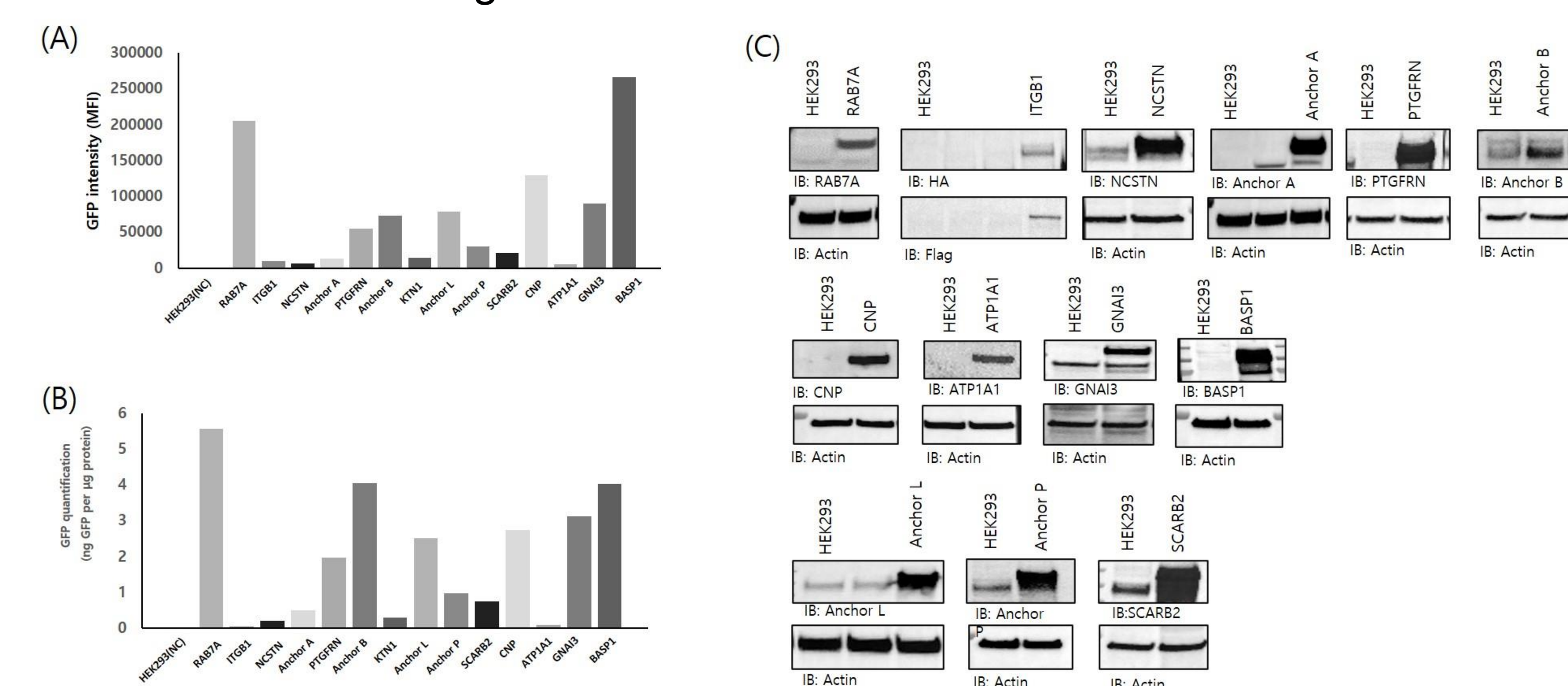


Figure 2. Overexpression of anchor candidates in stable cell lines. Overexpression of anchor fusion proteins was assessed by measuring fluorescence intensities (A) and quantifying GFP expression (B). (C) Immunoblot analyses confirmed their overexpression.

Summary & Future Prospects

- CDV engineering requires stable and efficient anchor proteins, which are specific and abundant in CDVs.
- Proteome analysis provided a deep understanding of the subcellular origin of CDVs and helped to find a list of membrane proteins that could be anchor proteins for HEK-CDV engineering.
- We evaluated anchor candidate proteins and chose a set of HEK-CDV anchors located in the plasma membrane or the lysosome.
- Anchor proteins will be bases for the genetic engineering of HEK-CDVs to introduce targeting ligands and therapeutic cargos to BioDrone therapeutics.

Identification and Characterization of BioDrone Anchor

We analyzed the amount of anchor fusion proteins in the CDVs and the distribution at a single particle level to evaluate anchor proteins for HEK-CDV BioDrone (The star indicate the selected anchors according to the anchor GFP fusion protein level, the ratio of GFP (+) particle and the number of GFP molecules on CDV as well).

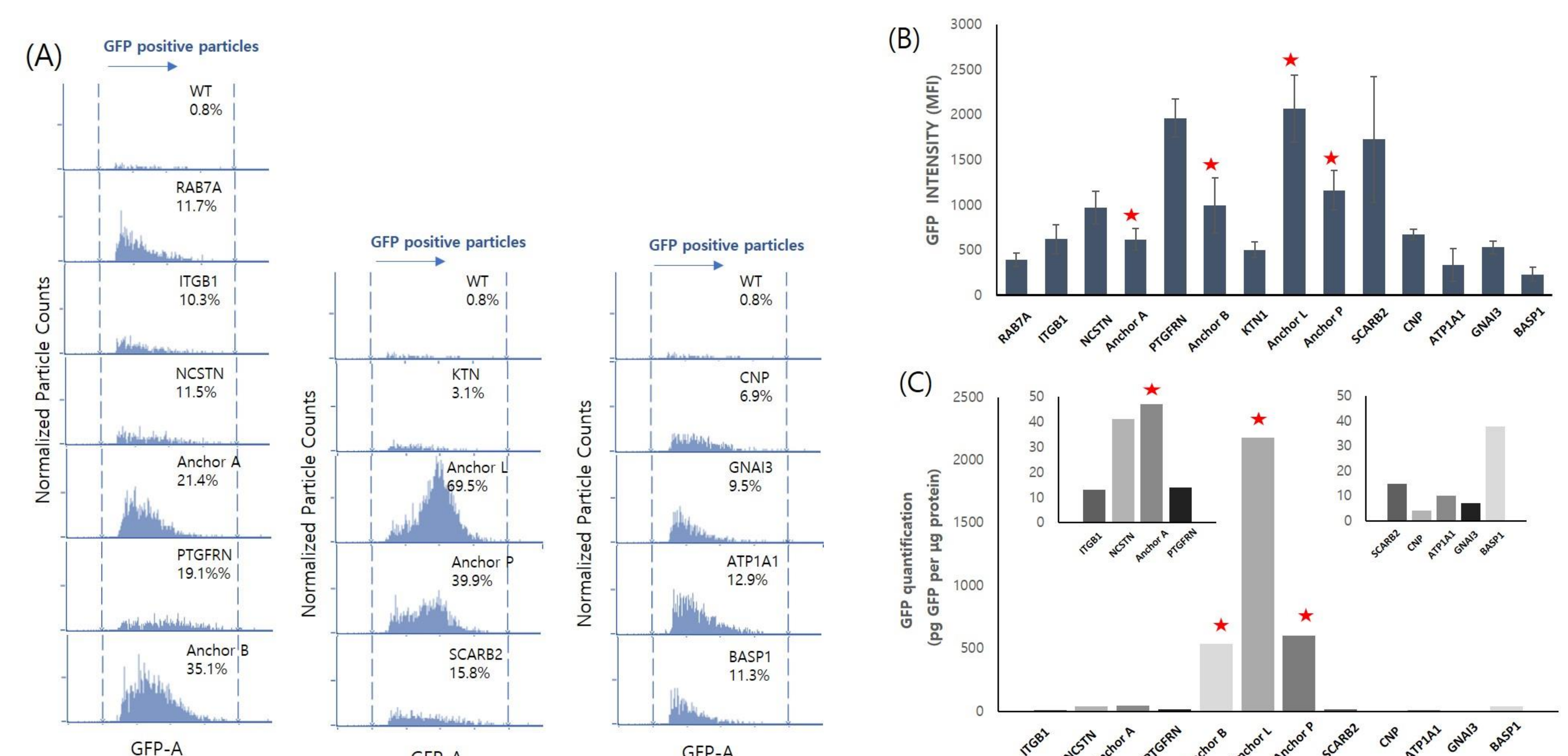
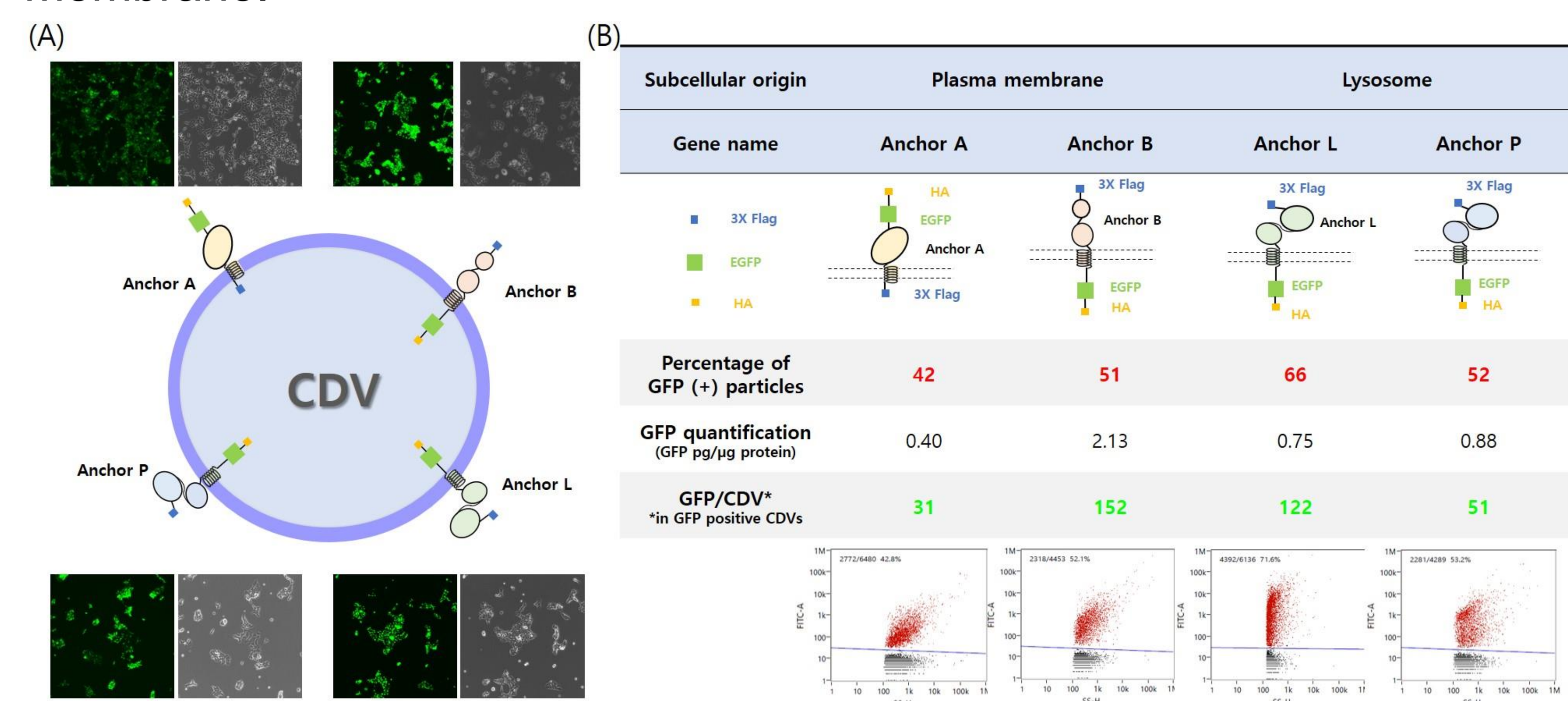


Figure 3. Characterization of anchor proteins in HEK-CDV

(A) Representative histogram from nFCM measurements of GFP positive particles. GFP intensity (B) and quantification (C) of HEK-CDVs expressing anchor-GFP fusions.

Selected BioDrone Anchors for HEK-CDV

We finally determined four BioDrone anchors which are abundantly distributed on CDV membrane.



Subcellular origin	Plasma membrane		Lysosome	
Gene name	Anchor A	Anchor B	Anchor L	Anchor P
Percentage of GFP (+) particles	42	51	66	52
GFP quantification (GFP pg/µg protein)	0.40	2.13	0.75	0.88
GFP/CDV* *in GFP positive CDVs	31	152	122	51

Figure 4. Selected anchors for BioDrone platform. (A) Schematic diagram of selected anchor proteins and their GFP fluorescence in HEK293 cells. (B) Characterization of selected anchor. The table shows GFP (+) particles and GFP protein number per CDV as well.

Comparison of BioDrone Anchor – CDVs from Different Extrusion Methods

We compared the characteristics of BioDrone anchors in the CDVs from different extrusion methods (membrane filter and large-scale extrusion). The selected anchors were high in both CDVs.

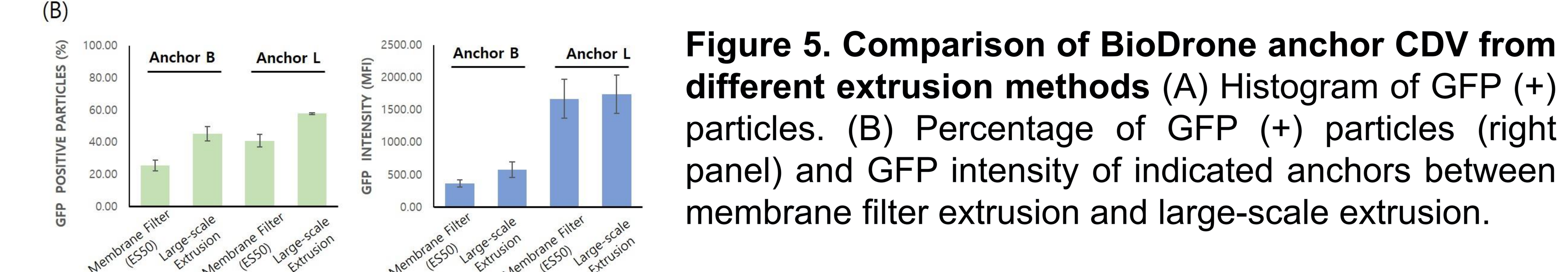


Figure 5. Comparison of BioDrone anchor CDV from different extrusion methods (A) Histogram of GFP (+) particles. (B) Percentage of GFP (+) particles (right panel) and GFP intensity of indicated anchors between membrane filter extrusion and large-scale extrusion.

Topology Analysis of Selected BioDrone Anchor

CDVs were treated with proteinase K (PK) to define the topology of selected anchor proteins. Since PK cannot penetrate through the lipid bilayer, only the surface parts of the anchor proteins are susceptible to PK degradation. The results ensured that CDVs maintained the topology of anchor proteins.

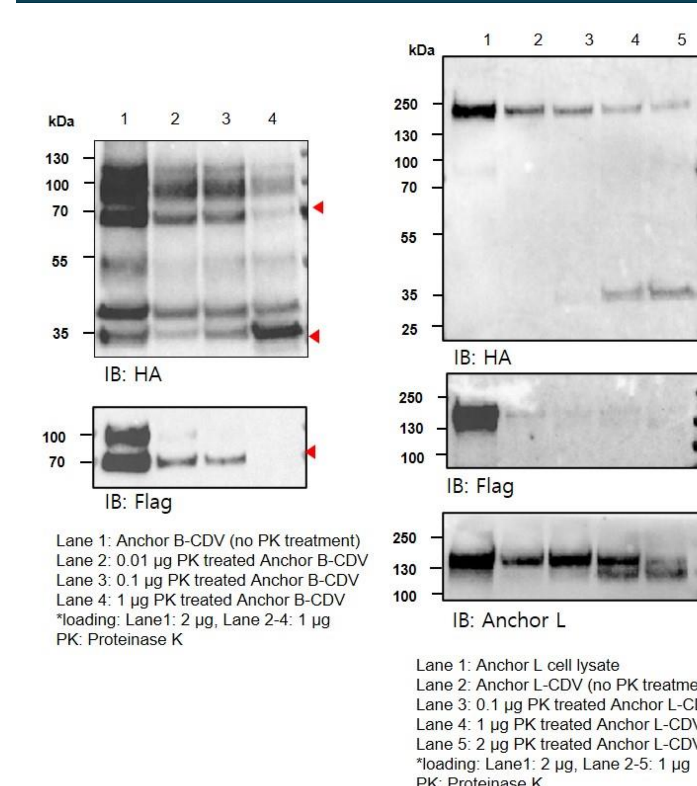


Figure 6. Topology analysis of anchor proteins via protease cleavage assay. BioDrone anchor CDVs were treated with various concentrations of PK and analyzed through western blot. HRP conjugated anti-Flag or anti-HA were used for detection of 3x Flag tag at the N-terminal and HA tag at the C-terminal of the anchor proteins.