学位論文内容の要旨
（Abstract of thesis）

博士の専攻分野の名称　博士（医理工学）　氏 名
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学 位 論 文 題 名

（Title of thesis）

Delivering Functional Cargos by Extracellular Vesicles Engineered with a Lipid-binding Domain

(脂質結合ドメインを用いた人工改変細胞外小胞による機能的カーゴの送達)

[Background and Objectives]

Custom-designed drug delivery vehicles for biomedical applications now predominantly involve the use of extracellular vesicles (EVs) as opposed to viral vectors. EVs represent an array of lipid bilayer membrane derived vesicles containing diverse cargos including DNA, RNA, proteins, lipids, and metabolites. EVs distinctive features make them an attractive candidate for intracellular and targeted protein delivery. Majority of the engineering approaches for loading exogenous bioactive molecules into EVs has been through the ubiquitously expressed tetraspanins CD9, but CD9 is believed to be involved in viral infection and may also affect precise targeting of cells. In this thesis, we report an alternative platform for recruiting protein of interests (POIs) into EVs through a lipid-binding domain 4 (D4). We studied the efficiencies of D4 for EV loading and for intracellular protein delivery.

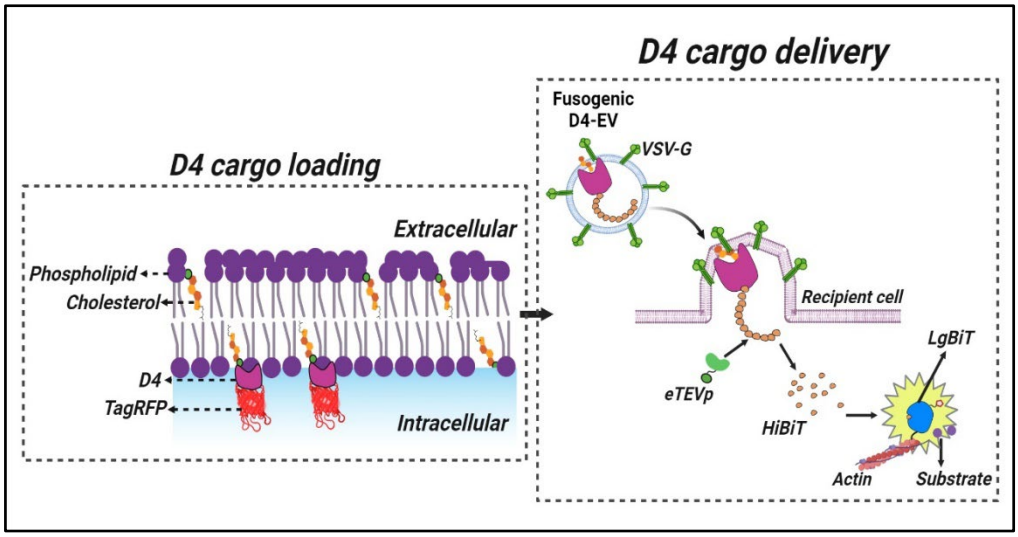

Fig I: Overall schematics of this study

[Methods]

1| We constructed plasmid vectors expressing several fusion proteins such as TagRFP, FusionRedMQV (FRMQV) and mScarlet-I as models for POI and individually fused them to EV sorting proteins to generate CD9-POI, D4H-POI, D4-YDA-POI, and D4-YQDA-POI.

2| We evaluated the protein expression and intracellular localization of these plasmid vectors after stable transfection in various cell lines. D4 efficiently recruits POI into EVs and shows localization to plasma membrane (PM) and vesicular structures in cell cytosol.

3| We increased the affinity of D4 to cholesterol by introducing additional mutants into D4. Accumulation of D4 mutants was expressed in EVs and localizes to plasma membrane of cells.

4| Through immunoblotting, we calculated the loading efficiency of POI between D4 and CD9 and confirm that D4 loads POI with analogous efficiency as CD9.

5| With live imaging through confocal imaging, we showed that D4-EVs are internalized by recipient cells and are functional in cell's cytosol.

6| Lastly with the help of split-luciferase technology and fusogenic proteins, our analysis shows that D4-EVs are capable of intracellular protein delivery in recipient cells.

[Discussion]

In this thesis, we describe how a lipid-binding domain 4 (D4) derived from bacterial protein, *Clostridium perfringens* can be utilized as an active platform for loading exogenous cargo into EVs.

1| D4 have been previously reported to mediate cholesterol recognition and membrane binding. Since EVs are also enriched in cholesterol, we harnessed D4 binding affinity to cholesterol to facilitate engineering of EV cargo. Further studies are required to optimize D4's affinity to cholesterol for enhancing and regulating the loading of POI into EVs.

2| The uptake of D4-EVs was unexpectedly lower when compared with CD9-EVs. However, the efficiency of intracellular protein delivery between CD9 and D4 are almost equivalent when assisted with VSV-G. This suggest that lower uptake of D4-EVs does not affect protein delivery, however, further studies are required to investigate the effect of small molecule inhibitors and enhancers on the cargo delivery of D4-EVs.

[Conclusion]

Conclusively, we report for the first time the role that D4 plays in EV engineering. Our results show that fluorescent and luminescent reporters can be loaded into EVs through D4 and achieve equivalent loading efficiency with widely used CD9. The outcome of this research has the potential to open a new vista of exploration for studying cell-cell communication through D4 engineered EVs.