Development of antibody-conjugated cell-derived vesicle for tumor-targeting

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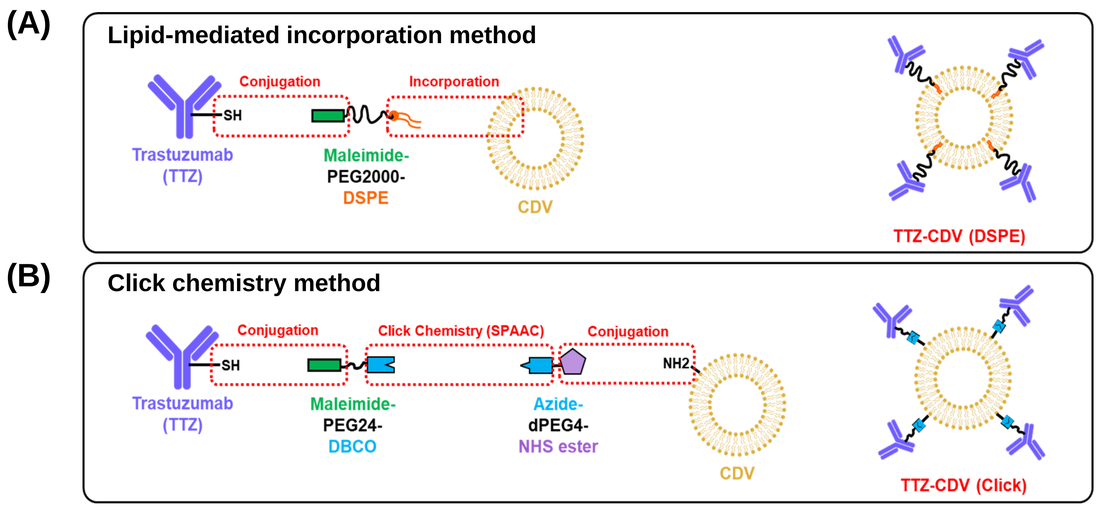
Introduction

Cell-derived vesicles (CDVs) are emerging as novel therapeutic candidates due to their similarity to extracellular vesicles and production scalability. To develop CDV as potent therapeutics, active targeting strategies are required to overcome its limited biodistribution. Affinity ligands to tumor-specific markers have been widely used as tumor-targeting moieties in nanoparticles. In this study, we utilized a well-developed therapeutic antibody as a targeting moiety, developed its conjugation method to the CDV, and evaluated the resulting antibody-CDV conjugate (AVC). Two different conjugation methods were assessed, click chemistry-based coupling reaction and lipid-mediated incorporation. First, an antibody was modified by DBCO-maleimide and CDV by NHS-azide. The DBCO-azide coupling reaction conjugated the resultant products. Second, an antibody was modified with 1,2-distearoyl-sn-glycero-3-phosphorylethanolamine (DSPE) and incorporated into the CDV membrane via the fusion of DSPE micelles. Both chemical modifications effectively displayed antibodies on the CDV surface with trastuzumab (TTZ) as a model antibody.



Scheme 1.Two different conjugation methods.

Click chemistry method of conjugation

Lipid-mediated incorporation vs. Click chemistry conjugation

Method	Lipid-mediated	Click chemistry
Time required	2h	5h
Number of TTZ per CDV	to 200	100 to 200
Characteristics	• Fusion of DSPE micelles and CDVs • No need for chemical modification of the CDV surface • Versatile in controlling the number of introduced antibodies • Able to introduce two antibodies at the same time	• Need modification of amine group on the CDV surface • Difficult controlling the number of introduced antibodies

Effect of PEG linker length on target binding

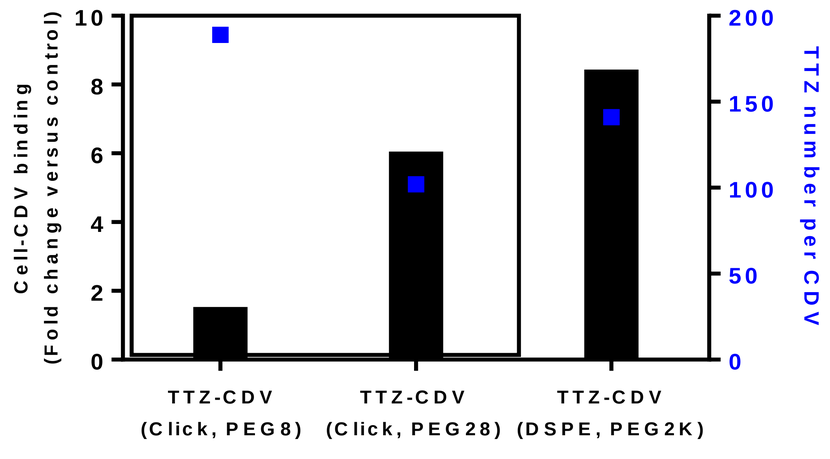


Figure 1. Relative binding of antibody-CDV conjugates to the target cells. Two antibody-CDV conjugates with different PEG lengths, TTZ-CDV(Click, PEG8) and TTZ-CDV(Click, PEG28), were prepared by click chemistry method and TTZ-CDV(DSPE,PEG2K) by lipid-mediated incorporation.

The length of the PEG linker was an important factor on binding of the antibody to the target cells. The TTZ-CDV of PEG28 with 102 TTZs on the CDV surface showed higher target binding compared to the TTZ-CDV of PEG8 with 189 TTZs.

Lipid-mediated incorporation

Characteristics of Lipid-mediated TTZ-CDV

TTZ-CDVs made by lipid-mediated incorporation were analyzed by nanoflow cytometry to assess TTZ (+) CDV, which showed that about 76 % of CDVs were decorated with TTZ. Furthermore, the amount of TTZ introduced into the CDV correlated to the concentration of TTZ-DSPE input in the incorporation reaction, implying a possible way to control the number of TTZ per CDV.

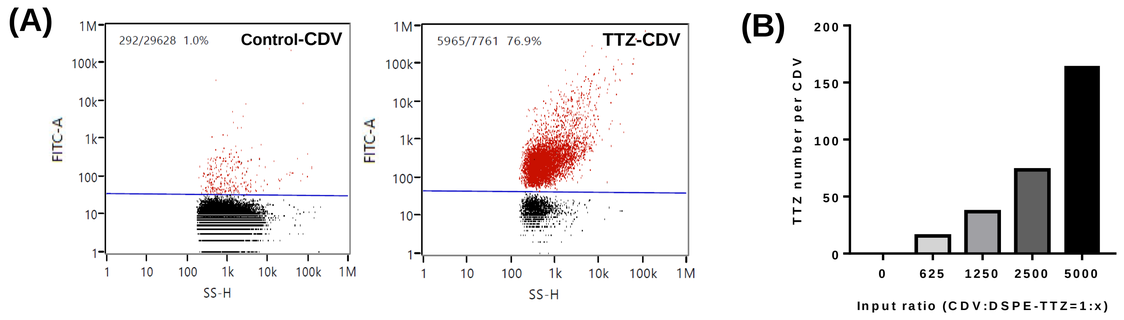


Figure 2. Analysis of TTZ-CDV. TTZ-CDV was immuno-stained using FITC-conjugated Fab fragment of anti-human IgG antibody and analyzed using nanoflow cytometry. (B) TTZ quantification in TTZ-CDV from various incorporation conditions. X means a relative molar number of TTZ-DSPE input.

Applicability of antibody incorporation method

A test of the simultaneous introduction of two antibodies into CDVs showed that 113 RTXs (rituximab) and 63 TTZs were introduced per CDV. The degree of DSPE modification was also an essential factor in antibody incorporation.

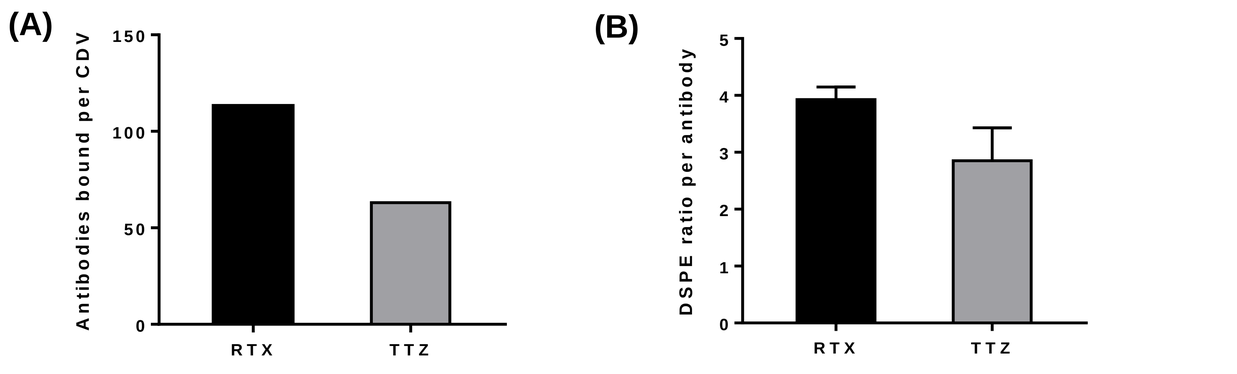


Figure 3. Simultaneous introduction of two antibodies. (A) The number of antibodies bound to one CDV in RTX-TTZ-CDV conjugate. (B) DSPE modification extent of RTX and TTZ.

In vitro binding/uptake assay of TTZ-CDV

TTZ-CDV demonstrated the targeting ability. TTZ-CDVs showed 15-fold higher binding to BT-474 than naïve CDVs. Moreover, target binding and cellular uptake increased proportionally to the number of TTZ conjugated to CDV.

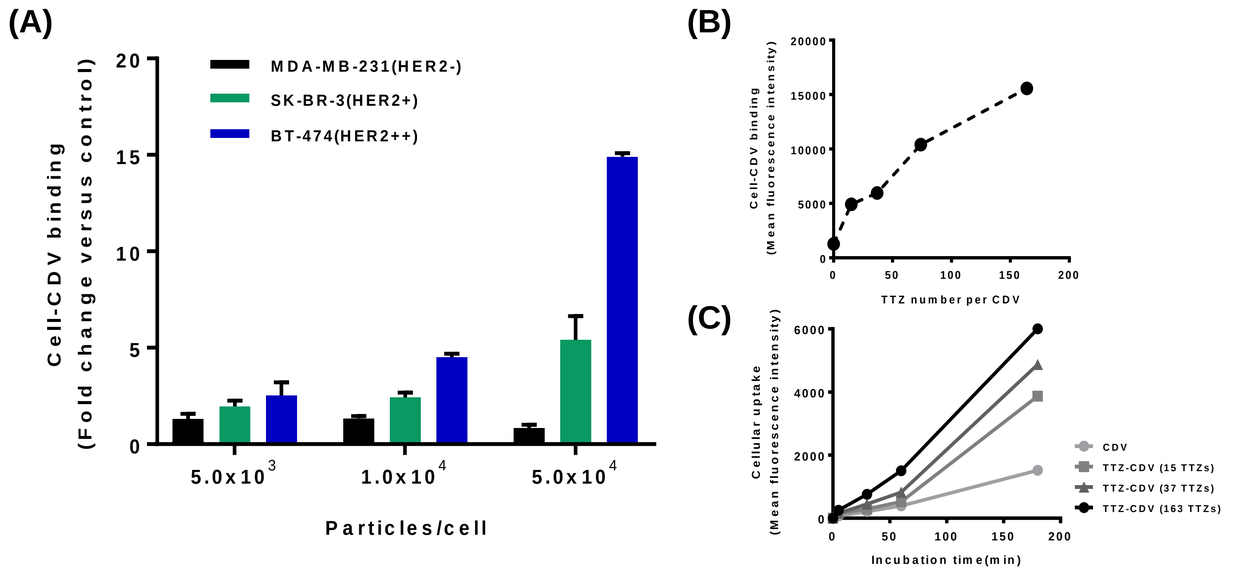


Figure 4. Binding and cellular uptake of TT-CDV. (A)TTZ-CDV binding to breast cancer cell lines, MDA-MB-231, SK-BR-3, and BT-474. TTZ-CDVs were prepared via lipid-mediated incorporation. (B) TTZ-CDV binding to BT-474 cells. TTZ-CDV was prepared with various amounts of TTZ-DSPE, which resulted in different amounts of TTZ in the CDV. The quantity of TTZ in the CDV determined their binding extent to target cells. (C) TTZ-CDV uptake of BT-474 cells. TTZ-CDVs were subject to BT-474, and their cellular uptakes were measured over time.

Summary & Conclusion

We applied antibody conjugation methods to CDVs to introduce targeting moieties. Engineered CDVs demonstrated the ability to bind to target cells, which led to improved cellular uptake. Currently, the evaluation of in vivo targeting of TTZ-CDV conjugates is in progress. This study will facilitate the development of potential BioDrones as tumor-targeting vehicles.