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Original Article

Production of *Escherichia coli* outer membrane vesicles displaying anti-MUC1 single chain variable fragment via SpyTag/SpyCatcher system

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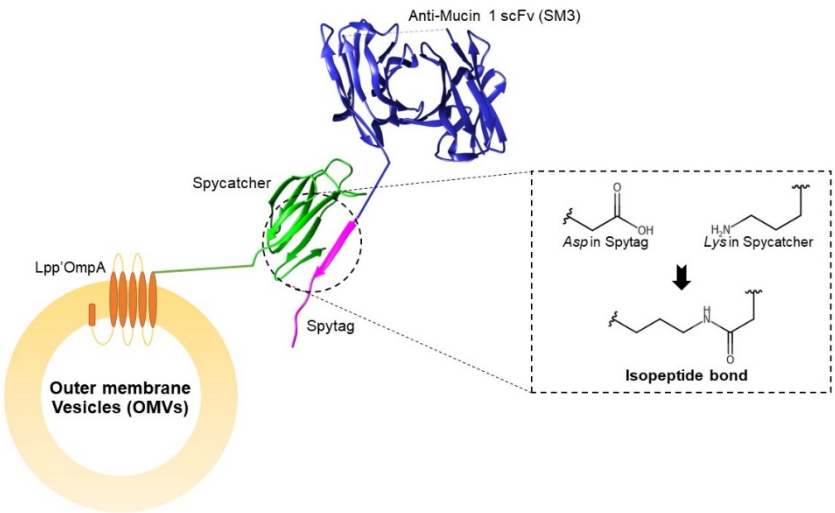
Abstract: Outer membrane vesicles (OMVs) are nanoparticles secreted from Gram-negative bacteria. Because of various properties of OMVs such as size, content, and simple production, bioengineered OMVs can be exploited as a vaccine/drug delivery system by developing OMVs to display desired proteins. Previous studies showed that proteins of interest must be genetically fused with outer membrane-localized proteins when inserting into OMVs. These proteins were produced in *Escherichia coli* hence they lack of correct post-translational modifications. To overcome this problem, we utilized the effective protein ligation system called SpyTag/SpyCatcher to attach foreign proteins from another expression system that retains their function on bacterial OMVs. SpyTag/SpyCatcher system enables coupling of two proteins through irreversible covalent bond resulting in extensive uses in surface decoration of various nanoparticles. In this study, we first produced SpyCatcher fused to Lpp'OmpA which is anchored on the surface of *E. coli* BL21(DE3)-derived OMVs. Then, SpyTag was fused to anti-Mucin 1 single-chain variable fragment and produced in ExpiCHO-S cells. After ligation of both components, Lpp'OmpA-SpyCatcher:SpyTag-SM3 complex was observed in Western blot analysis. It indicated that SM3 successfully displayed on OMVs. Thus, this ligation system will provide a robust and effective OMVs decoration.

Graphical abstract:



Keywords: outer membrane vesicle; spytag/spycatcher system; surface display system; vaccine platform; drug delivery system; mucin 1; lipoprotein; outer membrane protein a

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