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Development of sugar chain binding single chain variable fragment antibody to human papilloma virus (HPV)-infected cells for targeted therapy

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Abstract

Cervical cancer is a female specific cancer and it is considered to be caused by persistent infection of human papilloma virus (HPV). In 2012, 528,000 new cases of cervical cancer were diagnosed and 266,000 people were died worldwide. The prognosis is positive by the treatment in the early stage. However, the risk for biopsy in the diagnosis and side effects in vaccination or chemotherapy are burden for patients. Therefore, novel molecularly targeted reagent is strongly required for cervical cancer. In our group, to develop a molecularly targeted reagent, we focused on cell surface sugar chains because the expression levels and structures of cell surface sugar chains vary depending on cellular conditions including differentiation, inflammation, and concertation. Recently, we developed a cell surface sugar chain binding single chain variable fragment antibody (scFv) as a novel molecularly targeted reagent for adult T cell leukemia using our sugar chain-based nanobiotechnology and a phage display method[1]. In this study, we applied our technology to develop cell surface sugar chain binding scFvs for cervical cancer cells toward development of a novel therapeutic and diagnostic reagent.

In ca. 60-80% of patients of cervical cancer, part of HPV type-16 or -18 genes has been detected. Therefore, the genes, especially E6 and E7 in HPV-16 or -18, are biomarker for developing cervical cancer. In the present study, HeLa cells containing HPV-18 gene were used. First, HeLa cells were disrupted by homogenizer and sonication, and the cell membrane fraction was treated with Triton X-100. Then, the cell membrane proteins were separated by ultracentrifugation. The resultants proteins were treated with *N*-glycosidase F, and released *N*-linked sugar chains were captured and purified with a BlotGlyco® glycan purification kit. Purified *N*-linked sugar chains were conjugated with our original linker molecule. Obtained sugar chain ligand conjugates were analysed using MALDI-TOF/MS and several mass spectral peaks corresponding to *N*-linked sugar chain were detected. The conjugates were then separated into 4 fractions by HPLC using an ODS column. Each fraction was then immobilized on optical fiber to prepare fiber type Sugar-Chips[2]. The selectivity of the prepared chip was tested with a localized surface plasmon resonance (LPR) method and sugar chain binding proteins. Using the chip, a screening of scFv-displaying phages is now under investigation to obtain scFvs which bind to Hela cells.

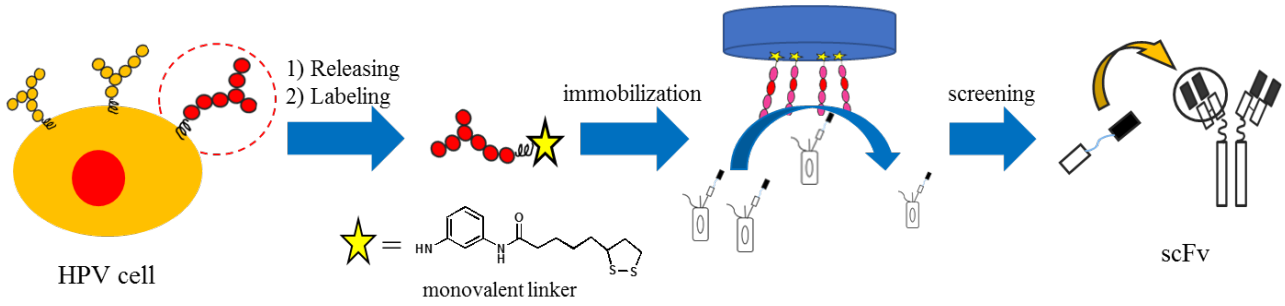

Figure. Schematic representation for developing cell surface sugar chain binding scFvs using fiber type Sugar Chip and a phage library.

Reference

[1] Muchima, K., et al. under revision. [2] Wakao, M., *et al. Anal. Chem.*, **89**, 1086-1091 (2017).

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