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Development of sugar chain binding single chain variable fragment antibody toward targeted therapy for adult T-cell leukaemia

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Abstract

Adult T-cell Leukaemia (ATL) is a blood cancer caused by the infection of retrovirus, human T-cell leukaemia virus type-1 (HTLV-1). Approximately 2.5-5% of HTLV-1 carriers develop ATL after an incubation period of more than 30-50 years. In Japan, HTLV-1 carriers are estimated to be approximately 1.08 million and are mainly distributed in the southwestern region of Japan including Kagoshima. Once a HTLV-1 carrier develops ATL, the prognosis is quite poor and 5-year survival rate is only ca. 10%. Specific mechanism for development of ATL has not been revealed and a standard therapy and diagnosis have not been established. In our group, to develop novel diagnostic and therapeutic drug for ATL, we focused on cell surface abnormal sugar-chain as a molecular target. Recently, three kinds of single chain variable fragment antibodies (scFvs), designated as S1TSCFR3-1, K33, and K34, were isolated from a phage library and a screening method with our Sugar Chip technology. However, the binding potencies of K33 and K34 to ATL cells are decreased in a month, indicating the low stability of the scFvs. On the other hands, S1TSCFR3-1 is very stable. In the present study, we addressed the preparation of chimera scFvs consisting of VH domain of S1TSCFR3-1 and VL domain of K33 or K34 using gene recombination.

Schematic image for the preparation of the chimera scFvs using gene recombination is shown below. pET-28b (+) DNA vector was used for a plasmid vector. A plasmid insert consisted of VH domain of S1TSCFR3-1 and VL domain of K33 or K34 were prepared using PCR. Because there is no common recognition sequence for restriction enzyme on the plasmid insert and vector, the recognition sequence for Hind III were added to the plasmid insert using TA cloning. After digestion of plasmid insert and vector using Hind III and Not I, they were ligated with ligase. The resultant plasmid was transformed into *E.coli* (BL21 (DE3) line) and several colonies were obtained. Then, the DNA sequence analysis revealed that the targeted sequence consisting of VH domain of S1TSCFR3-1 and VL domain of K33 were incorpotated into the plasmid. Preparation of the soluble scFv is now ongoing.

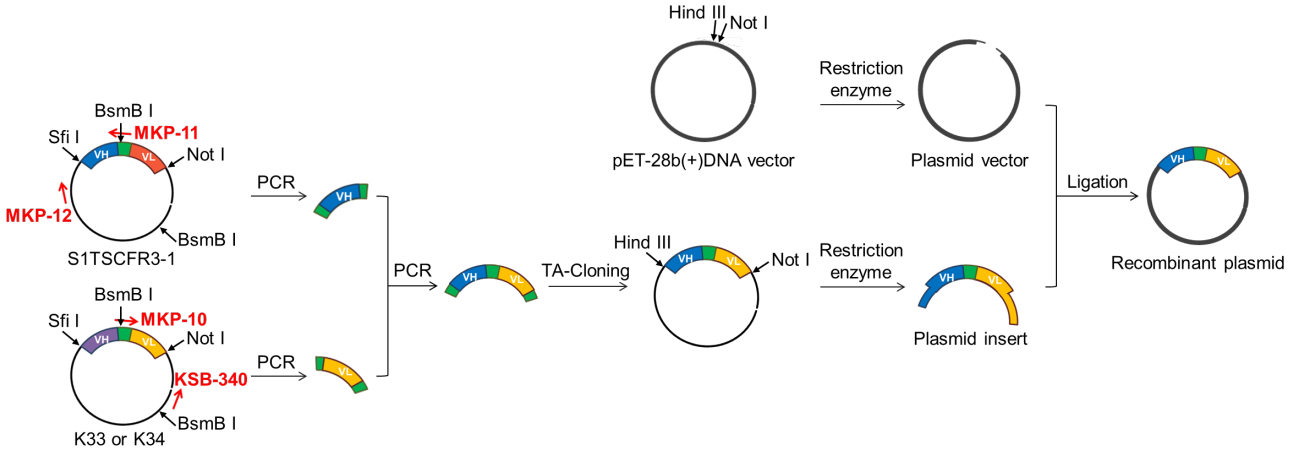

Figure. Schematic image for preparation of *O*-glycan binding scFv using gene recombination

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