Construction of recombinant vector harboring gene encoding scFv against EpEX and *Pichia pastoris* transformant isolation

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Introduction

As a transmembrane glycoprotein, the epithelial cell adhesion molecule (EpCAM) has been shown to be strongly overexpressed on the majority of tumor cells of epithelial origin and its overexpression has been supposed to support tumor progression and metastasis. Hence EpCAM has been made as a suitable antigen for targeted cancer therapy. In this case different types of antibodies including antibody fragments such as single chain fragment variable (ScFv) antibodies have been produced for drug delivery to this specific antigen. Pichia pastoris is a highly efficient and cost-effective system for expression of recombinant proteins. In this study, we used the Pichia expression system to express a ScFv against EpCAM extracellular domain (EpEX).

Materials and Methods: A codon optimized gene encoding anti-EpEX protein was cloned into the *Xho*I and *Xba*I sites of the pPICZαB vector. Transformation of CS115 strain was performed via electroporation method. The recombinant protein was linearized by using *Sac*I restriction endonuclease prior to gene integration into the genome.

Results: Successfully cloned anti- EpEX gene into the pPICZαB vector was confirmed by restriction analysis and sequencing. The transforming agents with genome containing inserted pPICZαB- anti- EpEX were confirmed via PCR amplification of genomic DNA using AOX1 primers.

Conclusion: These findings imply that the engineered strain is able to express the recombinant anti- EpEX which may be used as a potential candidate in cancer immunotherapy.

Key Words: EpCAM, Recombinant protein, ScFv, pPICZαB, *Pichia pastoris*, CS115