Designing and Engineering BiKEs For Expression In HEK293T cells

Puntaree Loveemongkol and Assoc. Prof. Dr. Aussara Panya [2]

[1] Student special project in B. sc. (Biology) student code 640510260, E-mail : puntaree_l@cmu.ac.th

[2] Department of Biology, Faculty os Science, Chiang Mai University, 239 Huay Kaew Rd, Suthep, Meung, Chiang Mai, Thailand, 52000, Email : aussara.pan@cmu.ac.th

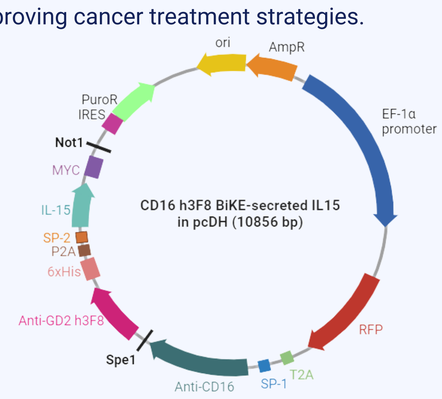
FACULTY OF SCIENCE
CHIANG MAI UNIVERSITY

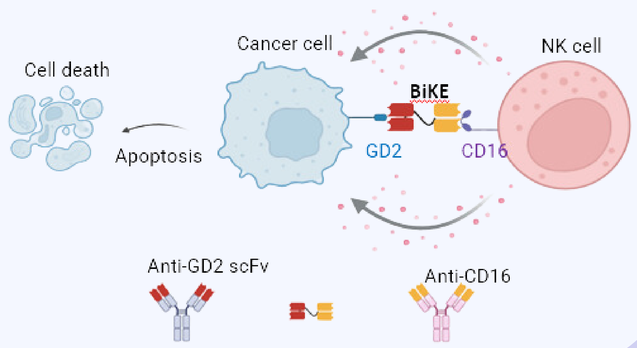
1 Abstract

Bispecific Killer Engagers (BiKEs) are innovative immunotherapeutic molecules designed to enhance immune cell-mediated cytotoxicity against cancer cells. In this study, we engineered a BiKE construct incorporating anti-GD2 and anti-CD16 targeting domains By simultaneously engaging CD16 on NK cells and GD2 on tumor cells, BiKE enhances NK cell-mediated cytotoxicity linked via a flexible SP-2 linker, along with a secreted IL-15 domain to enhance immune activation. The BiKE gene was cloned into a lentiviral vector for stable expression in HEK293T cells, incorporating fluorescent and affinity tags for protein detection and transfection efficiency assessment. Transfection efficiency was confirmed via fluorescence microscopy, while Western blot analysis validated protein expression using a HIS-tag. Our findings demonstrate robust BiKE expression, providing a foundation for further functional studies, including NK cell activation and cytotoxicity assays., further amplified by IL-15 stimulation. This study underscores the potential of BiKEs immunotherapies in targeting GD2-positive malignancies, such as neuroblastoma and melanoma, paving the way for further preclinical development.

2 Introduction

Bispecific Killer Engagers (BiKEs) are a novel class of immunotherapeutic molecules designed to enhance immune-mediated cytotoxicity against cancer cells. BiKEs function by simultaneously engaging tumor-associated antigens and activating immune effector cells, particularly natural killer (NK) cells, through CD16 interaction. This dual-targeting mechanism enhances antibody-dependent cellular cytotoxicity (ADCC), making BiKEs a promising therapeutic strategy for various malignancies.In this study, an Anti-GD2 BiKE-secreting IL-15 construct was engineered to target GD2-positive tumors, such as neuroblastoma and melanoma, while promoting NK cell proliferation and activation through IL-15 secretion. The construct was cloned into a CD47 TriKE lentiviral vector, which provides stable expression and efficient immune activation. The vector was designed with His and Myc tags for detection and monitoring, and transfection efficiency was assessed in HEK293T cells using fluorescence microscopy. Protein expression was analyzed via Western blot, while sequencing validation was performed to ensure correct integration of the insert. The findings from this study contribute to the development of next-generation BiKE-based immunotherapies, with potential implications for improving cancer treatment strategies.



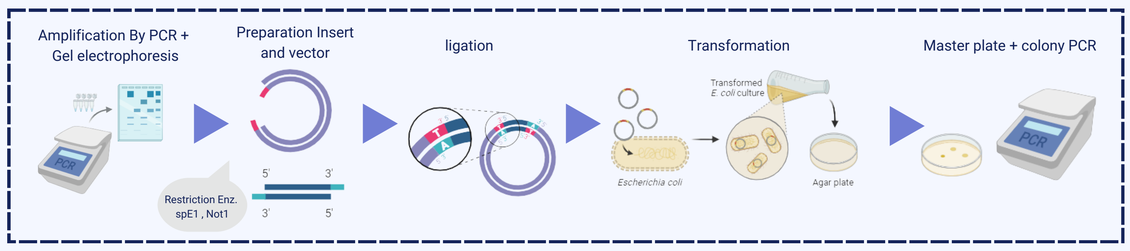


2 Objective

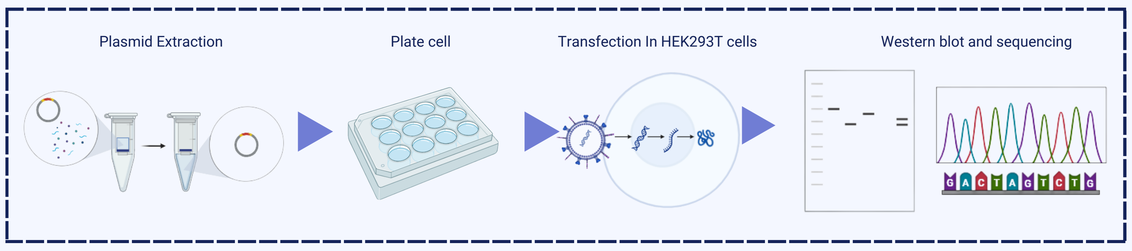
1. To design and construct the lentivirus vector encoding for anti-GD2 BiKE protein
2. To determine the expression of anti-GD2 BiKE protein in mammalian cells.

3 Method

1 Construction of lentivirus plasmid expressing anti-GD2 BiKE

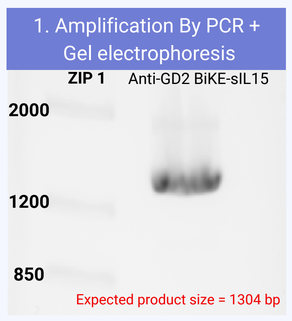


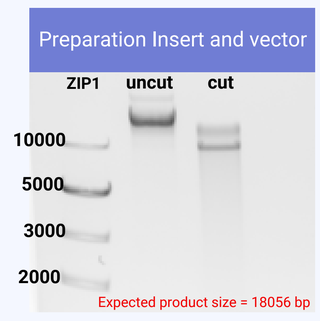
2 Determine anti-GD2 BiKE expression

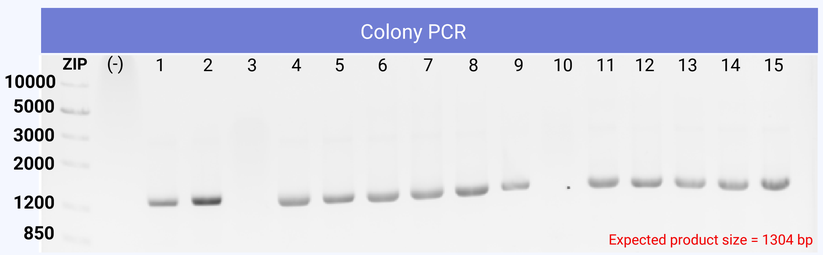


4 Result

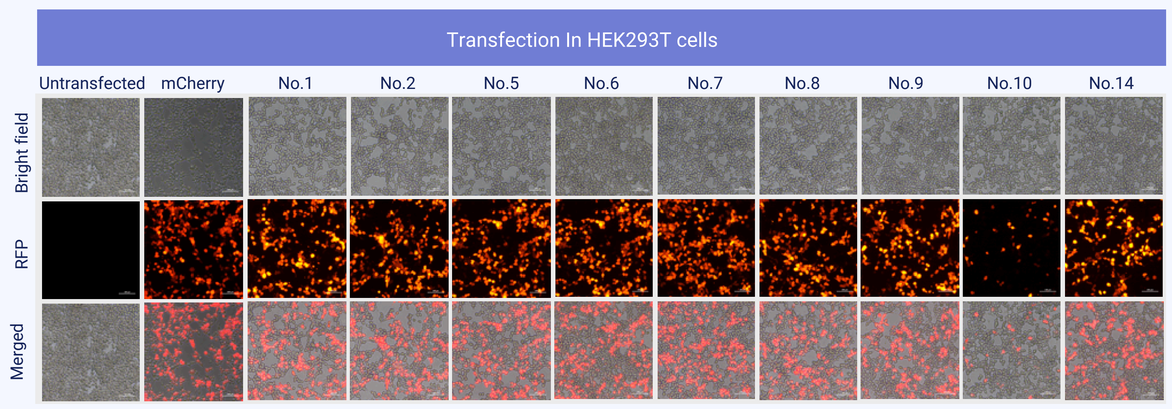
1 Construction of lentivirus plasmid expressing anti-GD2 BiKE

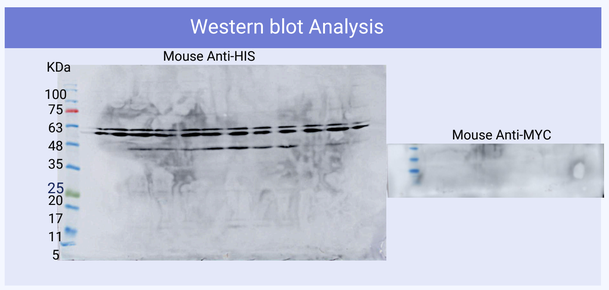


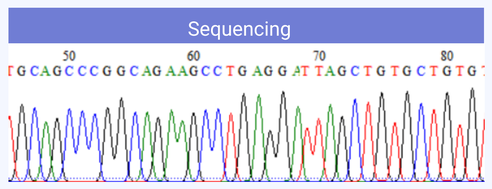




2 Determine anti-GD2 BiKE expression







5 Discussion

Western blotting, a reliable technique for protein quantification (Mahmood and Yang, 2012), confirmed the presence of BiKEs protein in HEK293T cells. The distinct protein bands observed indicated that the gene cloning strategy successfully facilitated proper protein expression. However, further analysis revealed a mutation in the SP2 domain, which is critical for directing protein entry into the endoplasmic reticulum (ER). Sequence analysis also identified a premature stop codon immediately after the 6His tag, likely resulting from a design error in the construct. This truncation prevents the translation of downstream sequences, including IL-15 and other essential domains. Consequently, the anti-Myc antibody is expected to fail in detecting the Myc tag in the Western blot, as the tag is located downstream of the premature stop codon and is not translated into the final protein product. This finding strongly correlates the lack of Myc detection with failed protein expression due to the erroneous sequence design. Overall, the study validates the effectiveness of the transfection process and highlights the utility of Western blotting in confirming protein expression in mammalian cells.

6 Conclusion

This study successfully developed BiKEs proteins but was unable to generate BiKEs that secrete IL-15. Despite this limitation, the findings provide a foundation for further optimization. If future research achieves successful cloning and secretion of IL-15, These BiKEs could be utilized in cancer treatment in humans.

Acknowledgment

I would like to express my sincere gratitude to Chutipa Chiawpanit, post-doc assistant, for teaching me cloning techniques and helping me develop both hard and soft skills. My heartfelt thanks to Assoc. Prof. Dr. Aussara Panya, my advisor, for her invaluable guidance and support. I also appreciate the support and insights from my fellow master's degree colleagues, which have been instrumental in this project.

Selected References

[1] Mahmood T, Yang PC. Western blot: technique, theory, and trouble shooting. N Am J Med Sci. 2012 Sep;4(9):429-34. doi: 10.4103/1947-2714.100998. PMID: 23050259; PMCID: PMC3456489.

[2] Nikkhoi SK. Design, engineering, and characterization of a bispecific killer cell engager with high affinity and specificity toward CD16a receptor on natural killer cells for cancer immunotherapy [dissertation]. New Brunswick (NJ): Rutgers, The State University of New Jersey; 2023.