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ANTI-EGFR^{VIII} ANTIBODIES AS A THERAPEUTIC AND DIAGNOSTIC CHALLENGE

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INTRODUCTION

Glioblastoma (GB) is a rapidly developing malignant brain tumor expressing the epidermal growth factor receptor variant III (EGFR^{vIII}). The mutated receptor may be a unique target of CAR-T (chimeric antigen receptors T cell therapy). Due to loss of 267 amino acids and appearance of a new glicine in the extracellular domain [1], EGFR^{vIII} has a unique epitope for the L8A4 antibody, which single-chain variable fragments (scFv) could be used in construct of anti-EGFR^{vIII} CAR in modified T cells.
CAR-T *in vivo* may result in serious side effects such as cytokine release syndrome (CRS). In the course of CRS, a rapid multiplication of CAR-T lymphocytes and sudden release of pro-inflammatory cytokines lead to extensive cytotoxicity and neurotoxicity. In would be desirable to develop a strategy to regulate the occurrence of CRS cytotoxic effects after administration of modified CAR-T cells to patient's body [2]. A regulation system based on appropriate agonists/antagonists of the transcription factor binding CAR promoter (introduced into modified T lymphocytes) may be an effective way to control the expression of chimeric antigen receptor in T cells.
The activities undertaken in the 2023-2024 academic year were aimed at verifying if developed heavy chain variable region fragment (VH) and light chain variable region fragment (VL) of L8A4 antibody specifically bind EGFR^{vIII} mutant, and obtaining plasmid vectors encoding: the anti-EGFR^{vIII} CAR sequence, the introduced binding sites of transcription factor, hereinafter referred to as TF2, or constitutive CMV promoter **(Fig. 1)**.

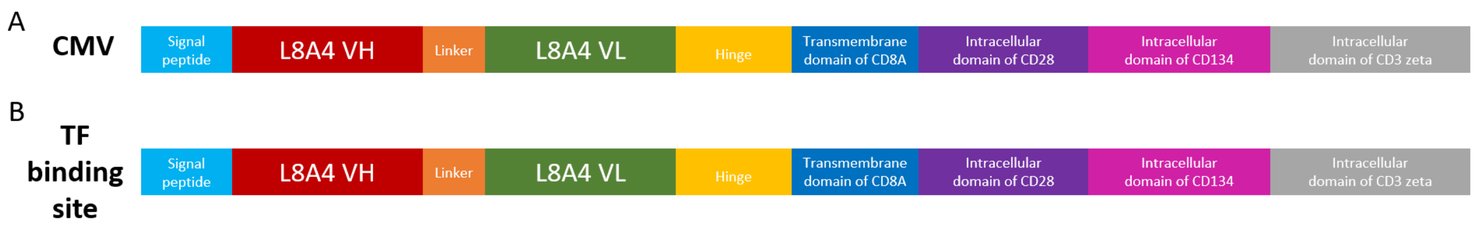

Fig. 1. Sequence of the third generation anti-EGFR^{vIII} CAR (containing the binding fragment of L8A4 antibody) in plasmids with constitutive CMV promoter (A) and introduced binding sites for TF2 transcription factor (B). *VH – heavy chain variable region fragment; VL – light chain variable region fragment; TF – transcription factor*

METHODS

ANTIBODY SPECIFICITY

Ordering plasmids encoding the sequences of L8A4 VH and VL.

Transfection of HEK-293T cells (24h or 48h) to produce the antibody using polyethyleneimine (PEI).

Collection of medium above the transfected cells for 5 days.

Isolation of the antibody using magnetic beads.

Western Blot analysis to compare the binding of EGFR^{vIII} by the produced antibody (anti-EGFR^{vIII} specificity) to commercial L8A4 (Absolute Antibody) in lysates of DK-MG^{high} and DK-MG^{low} glioblastoma cells.

VECTORS PRODUCTION

Transfer of the anti-EGFR^{vIII} CAR sequence to expression vectors using Gateway method.

Production of anti-EGFR^{vIII} CAR sequences flanked by attB sites in polymerase chain reaction (PCR).

BP reaction, entry clone propagation in competent bacteria in presence of zeocin and isolation.

Verification of plasmid sequence correctness *via* cutting the vectors with restriction enzyme, electrophoretic separation of the cut products and Sanger sequencing.

LR reaction, expression vectors propagation in competent bacteria in presence of ampicillin and isolation.

Verification of plasmid sequence correctness *via* cutting the vectors with restriction enzyme, electrophoretic separation of the cut products and Sanger sequencing.

RESULTS

Western Blot results confirmed that the isolated antibody binds the EGFR^{vIII} mutant similarly to the original L8A4 antibody (Absolute Antibody). The antibody does not bind the EGFR^{wt} (wild-type) **(Fig. 2)**.
Sanger sequencing confirmed the correct sequence of plasmid vectors encoding the anti-EGFR^{vIII} CAR under both the CMV promoter and TF2 binding sites **(Fig. 3)**.

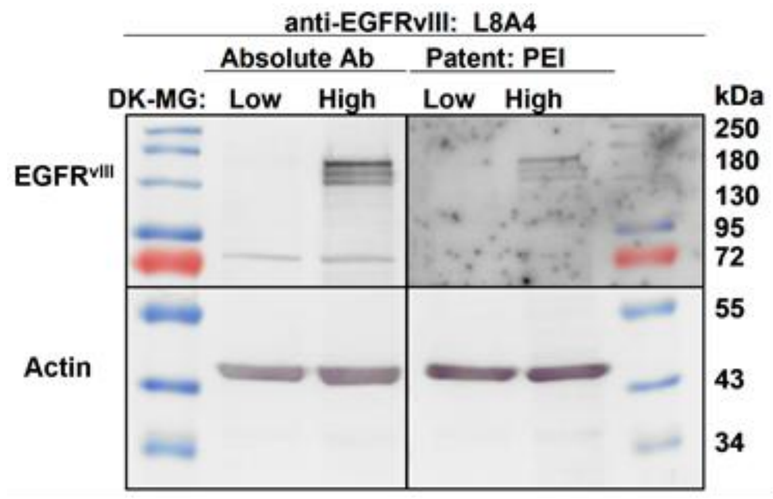

Fig. 2. Comparison of the commercial L8A4 antibody (anti-EGFR^{vIII}, Absolute Antibody) to the tested antibody based on the heavy and light chain variable region fragments (VH,VL) of L8A4 in DK-MG^{high} and DK-MG^{low} cell lines.

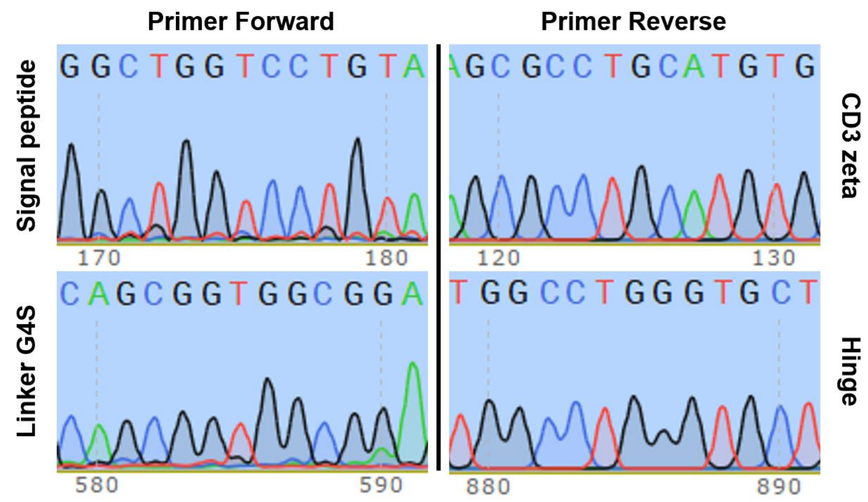

Fig. 3. Sanger sequencing of expression vectors encoding anti-EGFR^{vIII} CAR sequences (containing the L8A4 antibody binding fragment) with the constitutive CMV promoter or introduced binding sites for TF2 transcription factor.

CONCLUSION

The developed sequence of L8A4 VH and VL is specific for EGFR^{vIII}. The data suggest that using binding fragment of L8A4 in CAR-T cells may prove to be a promising therapeutic approach for treatment of glioblastoma expressing EGFR^{vIII}.

The sequences of genetically engineered plasmid vectors encoding CARs are consistent with the desired. Further analyzes need to be performed to obtain viral vectors coding anti-EGFR^{vIII} CAR with regulation system/constitutive promoter. The modified CAR-T cells must be tested for the ability to eliminate the population of EGFR^{vIII}-positive cells.

REFERENCES

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Scientific achievements and other activity directly related to implementation of doctoral dissertation
Publication:

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