BIO_06 - Expression of cetuximab biosimilar for development of Immunonanoparticle: Strategies and targets for drug delivery proposal towards treatment of prostate cancer

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Introduction: Biosimilar is a biologic product with the same efficacy and safety as an approved commercial reference. Cetuximab is a monoclonal antibody (Mab) inhibitor of epidermal growth factor receptor (EGFR). This mAb patent expired in 2016, because of it, the interest in developing its biosimilar for different application in treatment of cancer.

Objective: Expression and purification of monoclonal antibody anti -EGFR on the transient cell line for subsequent bioconjugation aiming drug delivery application.

Methodology: 1) Gene synthesis. The monoclonal antibody anti-EGFR development occurred with the synthesis of genes for variable heavy (VH) chain and variable light (VL) chain based on the commercial cetuximab sequence available on Drugbank. The, KOZAK sequence and signal peptide of human monoclonal antibody VLk e VH were added to gene construction. After that the genes were synthetised and inserted the into a vector pCDNA3.1. 2) Transfection and expression in transient cell line (Expi293f) with constructions VLk e VH. Cells were seeded at 2 x 10^6 cells/mL and co-transfected with a 15ug mixture of VH and VL DNA in the presence of lipofectamine (Expifectamin 293 Thermo fisher scientific ©) according to the Thermofisher scientific protocol. 3) Purification of monoclonal antibody. The antibodies were purified by affinity chromatography using protein G immobilized as a binder in AKta Purifier (GE) chromatography. 4) Enzyme-Linked Immunosorbent Assay (ELISA): An indirect and non-competitive ELISA based on the binding specificity of antibody anti -EGFR and cetuximab with the EGFR protein was carried out to test their biological activity.

Results: The genes for VH and VLk containing the CH1, CH2 and CH3 portions of human IgG1 were cloned into the expression vector pCDNA3.1 giving rise to the constructions pCDNA3.1 / VLk and pCDNA3.1 / VH. The expression of anti-EGFR antibody on reduced SDS-PAGE gel, shows band at ~50 kDa, ~25 kDa most likely represent VH chain and LV chain, respectively.

Conclusion: The gene construction strategy of the expression system pCDNA3.1 / VLk and pCDNA3.1 / VH demonstrated success with satisfactory expression level as preliminary experiment. In addition, the biosimilar mAb and Commercial Product present similar profile of binding to the EGFR protein. The expression of the complete monoclonal antibody (Mab) and characterization is ongoing in this moment. Moreover, the mAb is under optimization for improvement of protein yield.

Keywords: Biosimilar; Monoclonal Antibody Expression; Cancer