BIO.08 - Production of a scFv antagonistic to VLA-4 protein as a potential biopharmaceutical for chronic inflammatory diseases

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Introduction:

VLA-4 (Very Late Antigen 4, $\alpha4\beta1$ integrin) is a transmembrane heterodimeric protein present mainly on the surface of T lymphocytes and monocytes. As it mediates the transmigration of those cells from the endothelium to the inflammatory site, VLA-4 is critically related with most of the chronic inflammatory diseases, as multiple sclerosis and Duchenne muscular dystrophy. There is one therapeutic antibody, Natalizumab, able to recognize VLA-4, which is used for multiple sclerosis treatment. However, in some patients, this treatment might result in the recrudescence of a disease called progressive multifocal leukoencephalopathy. Besides, Natalizumab is also reported to interact with LPAM-1 ($\alpha4\beta7$ integrin). Therefore, Natalizumab is not totally safe. In this work, we search for the developed a scFv (*single chain variable Fragment)* able to specifically recognize VLA-4, employing *in silico* tools for the rational design of the molecule, recombinant technology for scFv production and *in vitro* functional assays.

Objective:

The aim of this work is to produce and evaluate a scFv specific for VLA-4.

Methodology:

The scFv was designed through molecular modelling, docking and dynamics and site-directed mutation. Then, the scFv was cloned in pET-28a plasmid and sequenced. It was expressed in *Escherichia coli* Shuffle strain. The expression conditions were optimized, the protein purification step was performed using immobilized metal ion affinity chromatography and purified protein was

detected and quantified. Enzyme-Linked Immunosorbent Assay (ELISA), transmigration and adhesion assays were performed using Jurkat T-Cell line, which is a VLA-4 expressing cell.

Results:

According to *in silico* tools, the interaction of the constructed scFv with VLA-4 was favorable, compared with the integrins VLA-5 and LPAM-1 The recombinant plasmid sequencing confirmed the nucleotide sequence corresponding to the scFv on the vector. The best condition for the scFv expression was growth in high cell density in Bioreactor with TB medium at 30°C and induction with 0.5mM IPTG for 4h, with a yield of 24mg/L of protein. It was expressed in inclusion bodies and then recovered and solubilized in buffer containing urea 8M. After chromatography and desalting, the final purity was higher than 90%. ELISAs showed that the scFv can recognize VLA-4 on the cell surface. Functional assays revealed that the scFv was able to promote cell adhesion on scFv-coated plates and reduce the cell migration in a fibronectin-driven *in vitro* transmigration, which is desirable for its application an inflammatory context.

Conclusion:

At present, a scFv designed as potential antagonist to VLA-4, produced by recombinant technology, revealed to be able to bind VLA-4 expressing cells and to partially inhibit VLA-4-mediated migration. As perspectives, new functional assays and complementary optimization steps will be performed, aimed at further assessment of this scFv as a potential new treatment for chronic inflammatory diseases.

Keywords: scFv; VLA-4; development