EVALUATION OF IRES-MEDIATED EXPRESSION AND DIFFERENT SIGNAL PEPTIDES FOR THE DEVELOPMENT OF CHO CLONES PRODUCING AN ANTI-PCSK9 MONOCLONAL ANTIBODY

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High levels of low-density lipoprotein (LDL) in the blood are associated to an increased risk of cardiovascular diseases, which are a leading cause of death worldwide. Despite this scenario, only minor progress was made since the introduction of statins as lipid-lowering synthetic drugs in the 1980s. Among patients suffering from high cholesterol levels, about 20% present strong adverse effects to statins or don't manage to decrease their LDL levels to normality. Since 2015, a new class of monoclonal antibodies (mAbs) was approved by FDA and EMA. These mAbs target the enzyme PCSK9 that is involved in the regulation of LDL cholesterol homeostasis, allow a higher amount of LDL receptors to be available on the surface of hepatic cells and thus enable a significant reduction of the circulating cholesterol levels.

In this study, we selected signal peptides and IRES elements from the literature to use for the construction of different tricistronic expression vectors. For the light chain, two different signal peptides were evaluated (SP1 and SP2), whereas for the heavy chain just one signal peptide was used (SP3). An EMCV IRES element was placed between the light chain (LC) gene and SP3. Downstream of the heavy chain (HC) gene, an attenuated IRES (att-IRES) element was inserted, followed by the antibiotics resistance gene for selection. In order to enable the evaluation of a stringent double selection (two different antibiotics) upon co-transfection with two plasmids, two sets of vectors were constructed, having either geneticin (neo) or hygromycin B as selection marker. Thus, a total of 4 vectors were constructed, having the following tricistronic cassette structure: CMV-IE promoter, SP1 or SP2, LC gene, EMCV IRES, SP3, HC gene, att-IRES, neo or hygro. CHO.K1 (ATCC, USA) previously adapted to suspension culture in TC-LECC medium (Xell AG, Germany) were transfected (or co-transfected) using Lipofectamine 3000 (Gibco, USA), using either circular or linearized plasmids.

Cell culture supernatants harvested 48h post transfection showed a similar expression level for all constructs. After approximately 2 months under selection pressure with the respective antibiotics, the stable cell pool that had been transfected with circular SP1-LC-SP3-HC-NEO was chosen for further studies. Cells were cultivated in shake flasks, attaining 13.9E6 cells/mL on day 7 and showing an specific growth rate of 1.2 d^{-1} during the exponential growth phase. The mAb was purified from cell culture supernatant using protein A affinity chromatography, showing high purity and homogeneity. Affinity of the purified mAb to PCSK9 was tested, confirming the success of the approach adopted in this work.