BIO 03 - Generation of a scFv antagonistic to VLA-4 integrin as potential therapeutic target in muscular dystrophies: *in silico* phase

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Introduction:

α4β1 integrins or VLA-4 are found in membranes of monocytes and T lymphocytes. Its participation in leukocyte migration is crucial for inflammatory diseases. Moreover, these proteins are also found in myoblasts and participate in the muscular regeneration process. Therefore, VLA-4 is an important therapeutic target for diseases such as muscular dystrophies, which are associated to the inflammatory process. ScFvs, Single Chain Fragment Variable, are fragments of antibodies which conserve the hipervariable regions of light and heavy chains, preserving their specificity. Currently, the only antibody commercially available, which interacts with α4β1 integrins, is Natalizumab. However, Natalizumab is not specific for α4β1. It also recognizes α4β7 integrins. Beside this, its application is not totally safe. In this context, this work aimed to build a scFv specific for α4β1 integrins through *in silico* tools and, later, *in vitro* tools.

Objective:

To design a scFv specific for VLA-4 through computational tools.

Methodology:

A search of monoclonal antibodies which recognize subunits α4 or β1 of integrins was done. From the selected sequences, the comparative modeling of antibodies' chains was done through Modeller program. Two types of scFv, using short and long linker, were made using the modeled chains. A molecular docking of all scFvs and α4β1 was performed through Haddock server. The best complexes obtained, according to Haddock parameters (Haddock score, Cluster size and RMSD) were submitted to Robetta Alanine Scaning for hotspot identification. From these results, mutations in strategic residues for interaction with α4β1 were done, using Coot program. Other dockings rounds using the modified scFvs and α4β1, α4β7 and α5β1 integrins were performed. The best scFv was selected and new mutations were done to ensure the antibody specifity for α4β1

comparing to the others integrins. In addition, Molecular Dinamics simulations were done to confirm the docking results and to analyze the main interactions between the scFv and VLA-4.

Results:

Three sequences were obtained from Integrity under 257898, 670484 and 725144 codes. The modeled chains were obtained and valuated. All modifieds scFvs showed a better Haddock Score for α4β1 integrin docking comparing to the originals antibodies. However, the best scFv was the modified 257898 one with short linker due to better Haddock score, Cluster size, and RMSD values. After the new mutations, this scFv also presented better docking parameters for α4β1 comparing to α5β1 and α4β7. Molecular Dynamics results ratified docking results about the scFv specificity and showed that salt bridges, electrostatic and Van der Waals interactions are determinant for the VLA-4 recognition by the scFv.

Conclusion:

A specific scFv for VLA-4 was obtained through computational tools and it can discern among α5β1, α4β7 and α4β1 integrins. As perspective, *in vitro* assays will be performed to authenticate the recognition and specificity properties of the scFv.

Keywords: scFv; Integrin; VLA-4