BIO_04 - Analysis of the c-Myc tag presence in the CAR's antigen-recognition domain structural stability, through molecular dynamics simulation

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Introduction: Chimeric antigen receptors (CARs) are recombinant proteins engineered to be expressed on the surface of cytotoxic lymphocytes to redirect their action in recognizing a specific epitope express on malignant cells, which is usually the CD19 protein. The CAR structure has a domain in the extracellular portion, which is commonly composed of a single-chain variable fragment (scFv), that is responsible for the antigen recognition. The scFv is formed by the light variable (VL) and heavy variable (VH) chains of a monoclonal antibody, which are connected by a linker peptide. The CAR detection can be made by adding a tag, as c-Myc, on its structure, like on the scFv. However, is necessary to evaluate if the c-Myc addition affects the scFv structural stability and if, consequently, it would affect that of the CAR as well, what can be done by employing technics such as molecular dynamics (MD) simulation.

Objective: Model the scFv structure to enable the construction of two systems, one only with the scFv and another with the c-Myc tag addition (c-Myc-scFv) to evaluate if the c-Myc incorporation will affect the scFv structural stability.

Methodology: The scFv tertiary (3D) structure was constructed with VL and VH derivated of the FMC63 antibody, which were connected with the linker (G4S)3, in the software Modeller 9.20 using the multiple templates protocol. The c-Myc (code PDB 2or9) was added to the scFv (c-Myc-scFv) employing the software PyMol. Each model was subjected to 700 ns of MD simulation in the GROMACS package, at the CHARMM36 force field, with the TIP3P water model, and a 0,15 M concentration of Na+ and Cl- ions. The scFv and c-Myc-scFv structural equilibrium was determined by Root Mean Square Deviation (RMSD).

Results: The RMSD shows that in the scFv system, VL-VH and VH achieves structural equilibrium after 250 ns and VL after 100 ns (RMSDs 0,16 ± 0,01, 0,17 ± 0,01 and 0,10 ± 0,01 nm, respectively) and in the c-Myc-scFv system, VL-VH and VH after 150 ns, and VL after 100 ns (RMSDs 0,16 ± 0,01, 0,15 ± 0,01 and 0,09 ± 0,01 nm, respectively). The linker was not considered in the RMSD analyzes due to its structural flexibility.

Conclusion: From the RMSD profiles, it was possible to observe that the c-Myc presence did not make the scFv structure unstable, but it has shown apparent stability and less structural modification compared to the beginning. According to this result, both scFvs can be used to study their interaction with the CD19 antigen, to analyze if c-Myc will interfere in the interaction between the structures. Lastly, the complete CAR structure will be constructed to analyze if will exist any difference arising from c-Myc presence.

Keywords: CAR; c-Myc; scFv