BIO_03 - Proposed mechanism for signal transduction of a CAR model in interaction with CD19, a cancer cell marker

Natália Fernandes Frota[1]*; Ana Júlia Ferreira Lima[1]; Alice Soares de Queiroz[2]; Marcos Roberto Lourenzoni[3].
[1]UFCe - Universidade Federal do Ceará;
[2]IFCE - Instituto Federal de Educação, Ciência e Tecnologia do Ceará;
[3]Fiocruz Ceará.

Introduction: Chimeric antigen receptors (CARs) are formed by three components: an extracellular domain, a transmembrane domain, and an intracellular domain. These receptors can be introduced into human T cells to redirect antigen specificity and improve function in passive immunotherapy. The extracellular domain is responsible for antigen recognition, which is usually formed by a single chain fragment variable (scFv); and a spacer (*hinge*). The main function of the transmembrane domain is to connect the extra and intracellular domains of a CAR and, as well as the hinge, may influence the CAR T cell effective function. The intracellular domain is the activating portion of T cells, usually formed of CD3-ζ. Immunotherapy using anti-CD19 CAR T cells is an effective treatment for B cells leukemia and lymphoma. CD19 is expressed on malignant B cells and is therefore a potent marker of cancer cells.

Objective: The objective was to obtain structural information, through molecular dynamics simulation, related to the signaling mechanism in a modeled CAR inserted into a T cell membrane model.

Methodology: The CAR components were modeled using molecular modeling and submitted to molecular dynamics (MD) simulations. The scFv, *hinge*, transmembrane and intracellular domain structures were connected to form the CAR and submitted to MD. MDs were conducted using the GROMACS 2018 and the force field used to describe the atomic interactions was CHARMM36.

Results: The distance, angle and PCA analyzes made it possible to infer a signal transduction mechanism in the CAR-CD19 system, which was not observed in the CAR system. The formation of the *hinge*-scFv interface and the approximation of this assembly to the membrane confers a reduction of the tension in the *hinge*-transmembrane binding region, which allows the inclination of the transmembrane α-helix bias. This inclination lasts from 370 ns to ~ 600 ns, at which time a conformational change in the intracellular domain is observed, ratified by the rapid transition observed on the PC1 curve at 600 ns. This sequence of events proposes an initiation of a signal transduction mechanism in the CAR T cell, which goes from the interaction between CD19 and scFv until the intracellular domain conformational, allowing CD3-ζ tyrosine residues to be phosphorylated by tyrosine-kinases inside the CAR T cell.

Conclusion: The movements leading to sequential conformational changes in CAR-CD19 are in agreement with the CAR function already described. The proposed mechanism ratifies conformational changes in the intracellular domain that are essential for exposing phosphorylation sites and initiate the function of CD3-ζ, which plays a role in signaling transduction.

Keywords: CAR T cell; CD19; Molecular Dynamics