BIO_12 - Obtainment and characterization of TIM-3_ECD-FC in HEK293-T cells as an antigen for the selection of antibodies for antitumor immunotherapy

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Introduction: T-cell immunoglobulin and mucin domain 3 (TIM-3) has emerged as crucial immune checkpoint receptor in the tumor microenvironment. The development of therapeutic strategies targeting TIM-3 holds great potential for enhancing antitumor immune responses. Despite the large amount of research developed about the TIM-3 role in the immune system, there is much contradictory evidence about specific ligand interactions and their relevance in the cancer perspective. Interaction inhibition of TIM-3 with its ligands by therapeutic antibodies showed promising results as an antitumor agent in preclinical and early-stage clinical studies. Previously we showed that recombinant TIM-3_ECD produced in bacteria provided expression gain of lymphocyte activation markers such as CD69, in activated human peripheral blood mononuclear cells (PBMC) showing a promising activation feature.

Objectives: The present work aims to clone, express and purify the extracellular portion of TIM-3 fused to the FC portion of antibodies in HEK293-T cells, to serve as antigens in the selection of blocking monoclonal antibodies that will be tested as possible immunotherapeutics.

Methodology: The synthetic pcDNA3.1-TIM-3_ECD-FC construction was transfected into HEK 293-T cells for transient expression, followed by the cell's supernatant protein G affinity chromatography to TIM-3_ECD-FC purification. The protein structure was first analyzed by SDS-PAGE and indirect ELISA, evaluating TIM-3_ECD- FC recognition by conformational specific anti-TIM-3 and anti-human Fc antibodies to confirm the molecule proper folding state.

Results: The purification yield was 1.3 mg/L. Purification was assessed by SDS-PAGE gel, displaying a single band at ~50kDa, suggesting high purity. By ELISA, it was possible to evaluate the correct conformation of the recombinant protein, once it was recognized by specific antibodies for TIM-3 and human FC. Functional analysis to evaluate its ability to modulate immune responses is ongoing.

Conclusion: Our partial results demonstrate the successful cloning, expression, and purification of functional TIM-3_ ECD-FC in HEK293-T cells. The molecule will be used to select antibodies of high affinity and therapeutic potential that can significantly increase the efficiency of immunotherapy.

Keywords: TIM-3; Recombinant protein; Antitumor immunotherapy