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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**. Interaction inhibition of TIM-3 with its ligands by therapeutic antibodies showed promising results as an antitumor agent. Previously we showed that recombinant TIM-3_ECD produced in bacteria provided expression gain of lymphocyte activation markers, in activated human peripheral blood mononuclear cells (PBMC) showing a promising activation feature.

METHODS

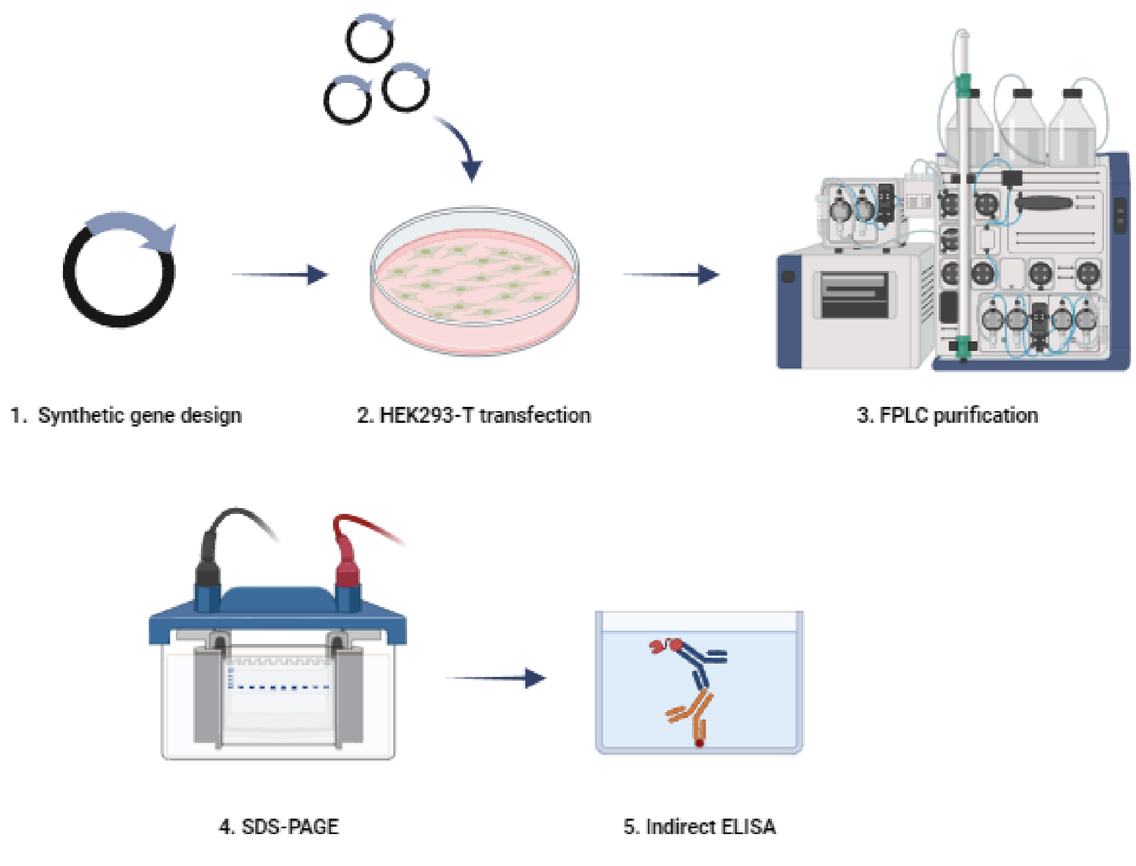

Figure 1. Methods. SOURCE: Created in BioRender.com

OBJECTIVES

Obtaining the recombinant purified version of TIM-3_ECD-FC in HEK cells for use as an immunotherapeutic tool and as an antigen for blocking monoclonal antibodies selection.

RESULTS

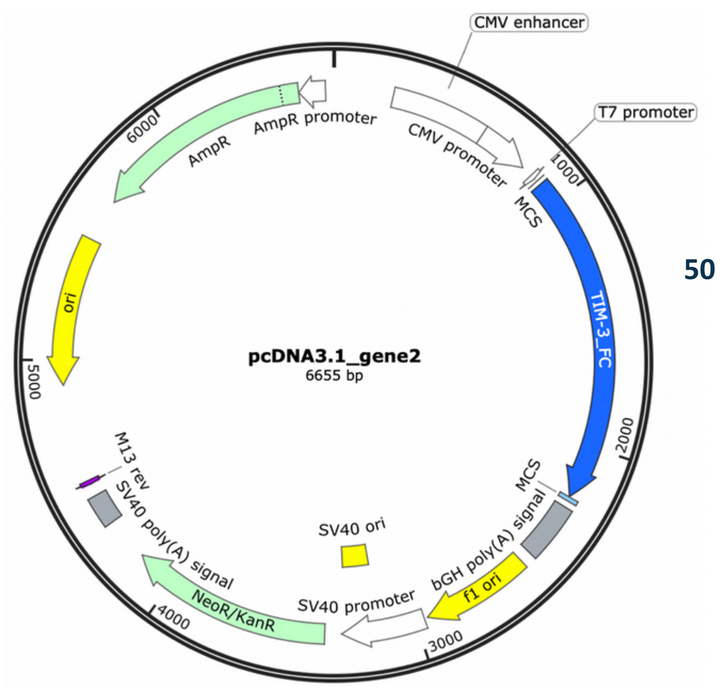

Figure 2. Schematic representation of the synthetic pcDNA3.1-TIM-3-Fc construction.

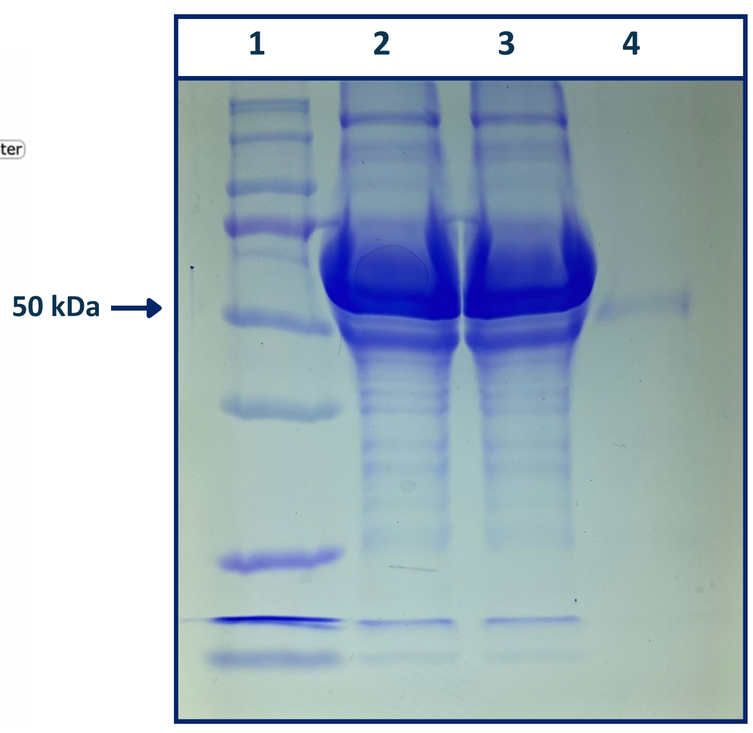

Figure 3. SDS-PAGE gel electrophoresis 12% of the purified recombinant protein TIM-3_ECD-FC. (1) Molecular weight marker Kaleidoscope (Biorad); (2) TIM-3_ECD-FC sample; (3) Flow TIM-3_ECD-FC; (4) TIM-3_ECD-FC eluate.

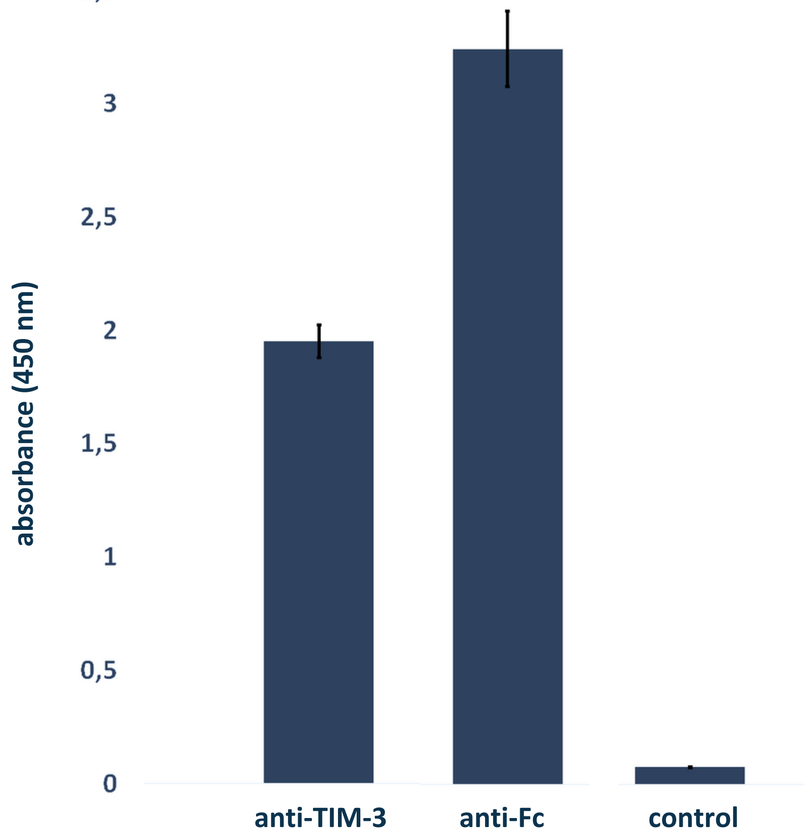

Figure 4. Indirect ELISA was performed in triplicate testing the detection of TIM-3_ECD-FC by anti-TIM-3 and anti-FC antibodies, using BSA as a negative control. Detection was performed using 5 µg/mL of antigen and the signal generated after incubation with mouse anti-IgG conjugated to peroxidase (1:10,000).

CONCLUSION

In order to obtain antibodies of high affinity and therapeutic potential that can significantly increase the efficiency of immunotherapy, **TIM-3_ECD-FC** was produced, its tests indicate the **appropriate folding state and the functionality** of the molecule, that will be used as an immunotherapeutic tool and to **select potential antibodies for immunotherapy.**

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