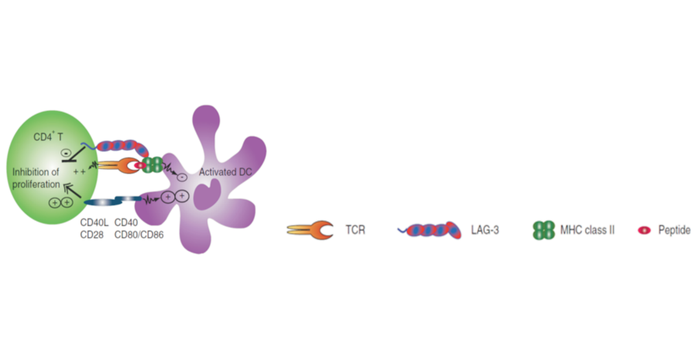
Screening of proteins related to the immunological checkpoint Lymphocyte activation gene-3 (LAG-3) through the BioID method

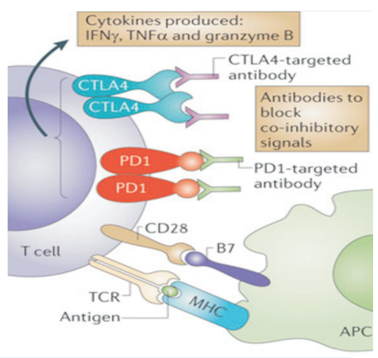
Priscila Rafaela Ribeiro (DO)[1], Marco Antônio Pretti[1], Leonardo Chycaibam,[1,2] Martin Hernan Bonamino[1,2].

1. Instituto Nacional do Câncer, INCA.
2. Fundação Oswaldo Cruz, FIOCRUZ.

INTRODUCTION

Inhibitory receptors, such as PD-1, LAG-3, TIM-3 and CTLA-4 have gained attention as potential targets for immunotherapy, once the manipulation of the negative signals mediated by these receptors may provide new therapeutic approaches for infectious diseases, transplantation, autoimmune diseases or cancer. More recently, CD-4 like lymphocyte activation gene-3 (LAG-3) was described as a cell surface molecule that interacts with high affinity through its cytoplasmic domain with MHC class II molecules. The identification of molecules that interact with inhibitory receptors is a key step to better understand the funcions of these receptors.





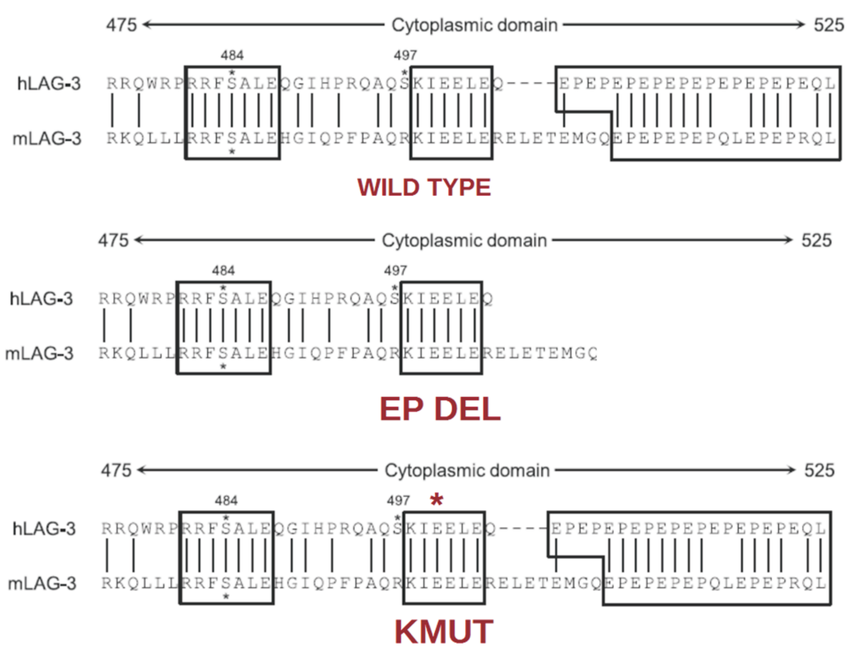
OBJECTIVE

The objective of this project is to conduct a screening of proteins that interact with LAG-3`s cytoplasmic domain by using the BioID method and identify the possible signaling pathways (in silico analysis) with which these proteins are involved, validate the presence of these proteins by western blot and / or flow cytometry.

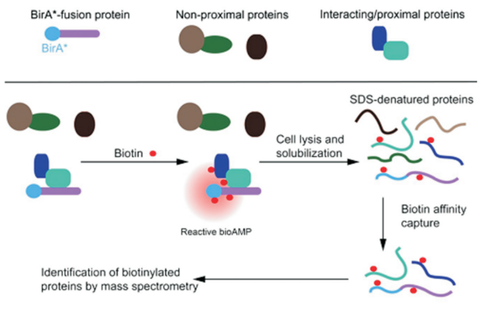
METHODOLOGY

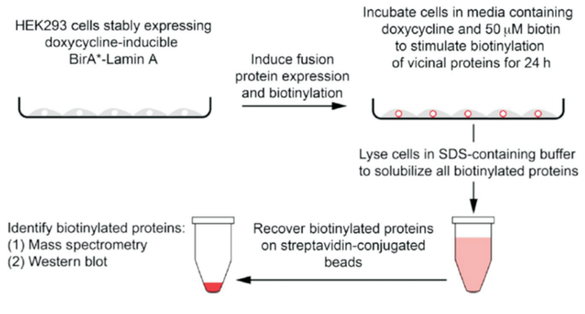
- Chimeric antigen receptors were built with extracellular domain scFv anti-CD20 (chimeric receptor antigen - CAR) containing the intracellular domain of Lag-3wild type, Lag-3 Kmut (mutation K => Non KIEELE) Lag3 EPdel (EP domain deleted) and Lag-3 Kmut EPdel (double mutant), all fused to the BirA Domain;

Construction of the chimeric antigen receptor (CAR)



Identification and quantification of proteins by mass spectrometry

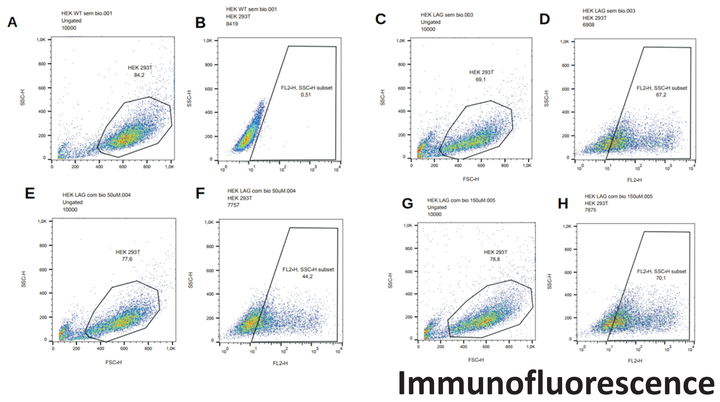




Roux et al., 2012

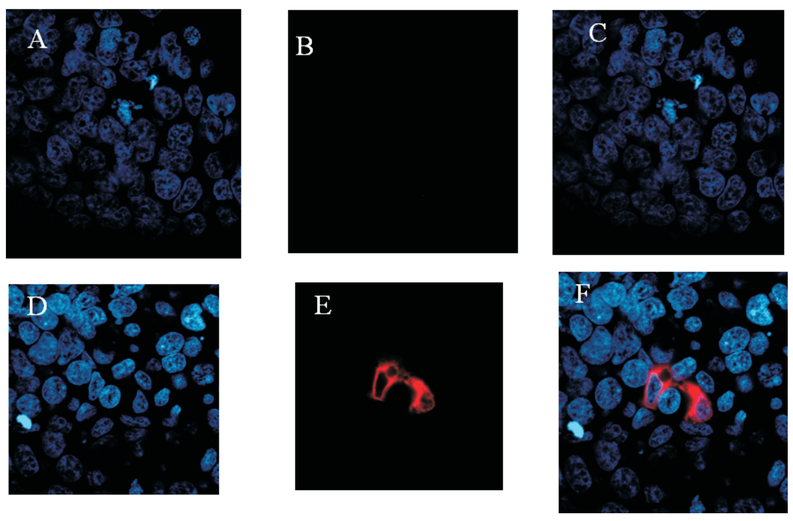
PRELIMINARY RESULTS

CAR 20LAG3BirA expression in HEK 293FT cells detected by flow cytometry



HEK 293FT cell line was electroporated with 10 µg of CAR 20Lag3 BirA and was incubated with primary antibody (anti-Fab 1:200) and secondary antibody (streptavidin APC 1:200) to detect the presence of the CAR. CAR expression were analyzed after 24h by flow citometry.
A) Gate of cells B) Cells stained just with secondary antibody- negative control for Streptavidin/APC; C) Gate of cells for D) Percentage of positive cells for SPV/APC, indicating expression of the CAR. E) Gate of cells for F) Expression of the CAR with 50uM added to the culture medium. G) Gate o the cells for H) Expression of the CAR with 150 uM added to the culture medium.

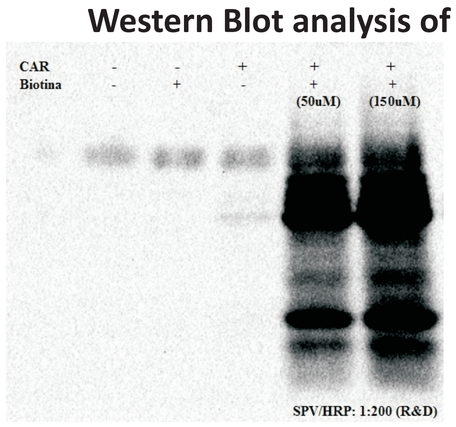
Immunofluorescence



For immunofluorescence, HEK 293FT cells were stained for DAPI (blue) and anti-HA antibody (1:30) followed by Alexa-Fluor 546 (1:250- red) and observed at confocal microscope.
All the pictures represents DAPI staining (blue) followed by the condition (Control, Lag3BirA, Epdel and Kmut) stained for HA antibody and Alexa Fluor 546 (red) and merge.

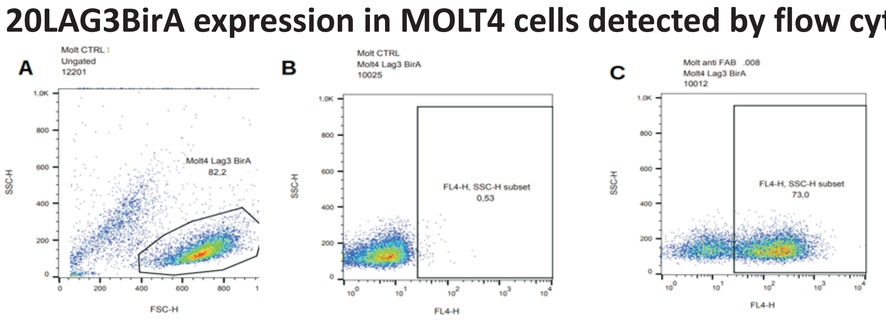
A-C: Control (not tranfected cells)
D-F: Lag3 BirA WT

Western Blot analysis of biotinylation in HEK 293T cells

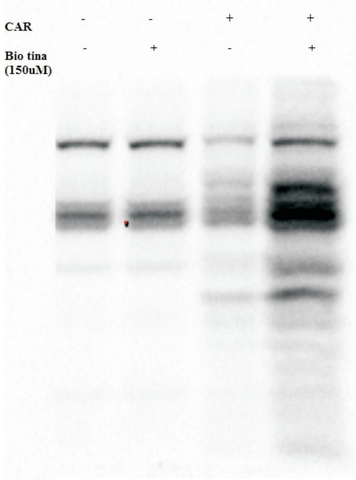


Western blot of HEK293 FT cells electroporated or not with CAR 20Lag3 BirA for biotinylation analysis.
The cells were grown under addition of excess of biotin to the cell culture medium (50 Mm OR 150uM final concentration).
Following SDS-PAGE separation, non transfected and 20Lag3 BirA cells were probed with streptavidin-HRP. The extensive biotinylation of proteins in the BioID-20Lag3 BirA conditions can be observed.

CAR 20LAG3BirA expression in MOLT4 cells detected by flow cytometry



Western Blot analysis of biotinylation in MOLT4 cells



Western blot of MOLT4 cells electroporated or not with CAR 20Lag3 BirA for biotinylation analysis.
The cells were grown under addition of excess of biotin to the cell culture medium (150uM final concentration).
Following SDS-PAGE separation, non transfected and 20Lag3 BirA cells were probed with streptavidin-HRP. The extensive biotinylation of proteins in the BioID-20Lag3 BirA condition can be observed.

Projeto Gráfico: Área de Edição e Produção de Materiais Técnico-Científicos / INCA