Screning of proteins related to the inhibitory receptor Lymphocyte activation gene-3 (LAG-3) through BioID method.

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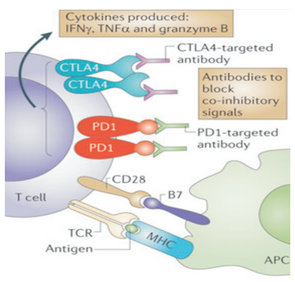
INTRODUCTION

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 both infectious diseases , transplantation, autoimmune diseases such as for cancer. More recently, CD-4 likelymphocyte 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. Recently, in addition to these inhibitory receptors, to identify which molecules interact with these has been the new goal of this area. Once identified, such molecules can also become possible new targets to be achieved. In 2012, in order to identify interactions between proteins dependent proximity shape and biotinylation, Roux et al (2012) developed a method called BioID, wich is based on the fusion of a protein of interest linked to a mutated biotinaligase (R118G) from Escherichia coli, wich is called BirA (CHOI-RHEE et al., 2004; Cronan, 2005; Roux et al, 2012.) This enzyme is able to biotinylate proteins associated with particular target protein. Once biotinylated, the proteins may be recovered by affinity (Kd = 10^{-14}) through beads conjugated to streptavidin and subsequently identified by mass spectrometry.



Speiser et al., 2011

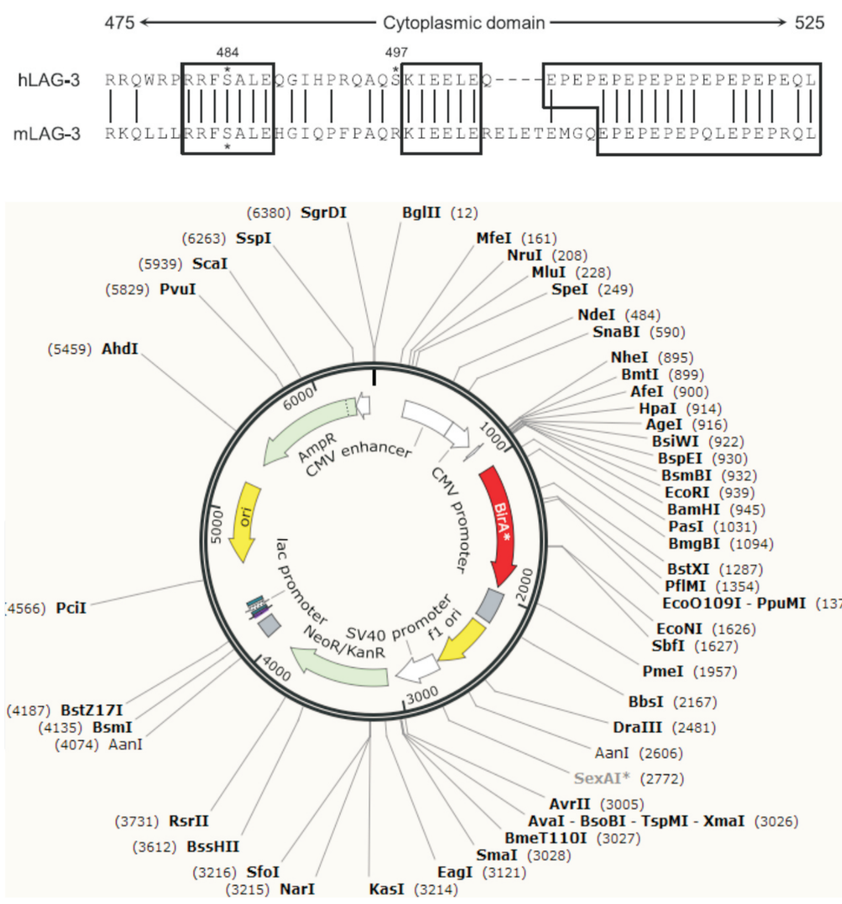
OBJECTIVE

The objective of this project is to conduct a screening of proteins that interact with LAG-3 by BioID method and identify the possible signaling pathways (in silico analysis) with which these proteins are involved, validate the presence of the same by western blot and / or flow cytometry and relate data obtained by increasing or decreasing the immune response by T cells.

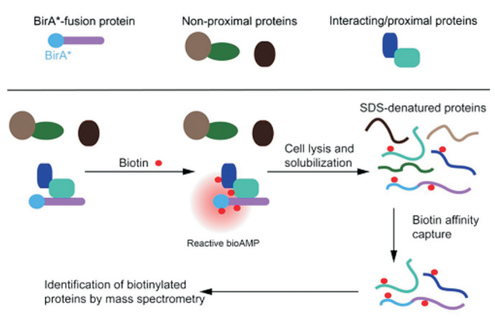
METHODOLOGY

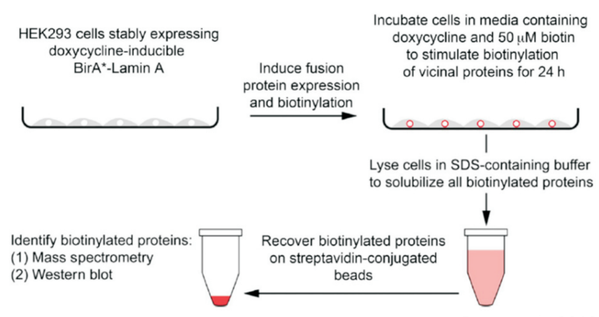
- To simulate the activation of LAG-3, we will be built chimeric receptors with extracellular domain scFv anti-CD20 (chimeric receptor antigen - CAR) containing intracellular domain of Lag-3wild type, Lag-3 Kmut (mutation K => Non KIEELE) Lag3 EPdel (EP domain deleted) and Lag-3 Kmut EPdel (double mutant), all fused to the BirA Domain;
- Subsequently inducing expression of these CARs cells in HEK293FT and CD4 + T lymphocyte Jurkat 20BBz +;
- Identification and Quantification of Proteins Associated to the inibithory receptor Lag-3 by mass spectrometry;
- In silico analysis of possible downstream signaling pathways wich lag3 is involved.

Construction of the chimeric antigen receptor (CAR)



Identification and quantification of proteins by mass spectrometry

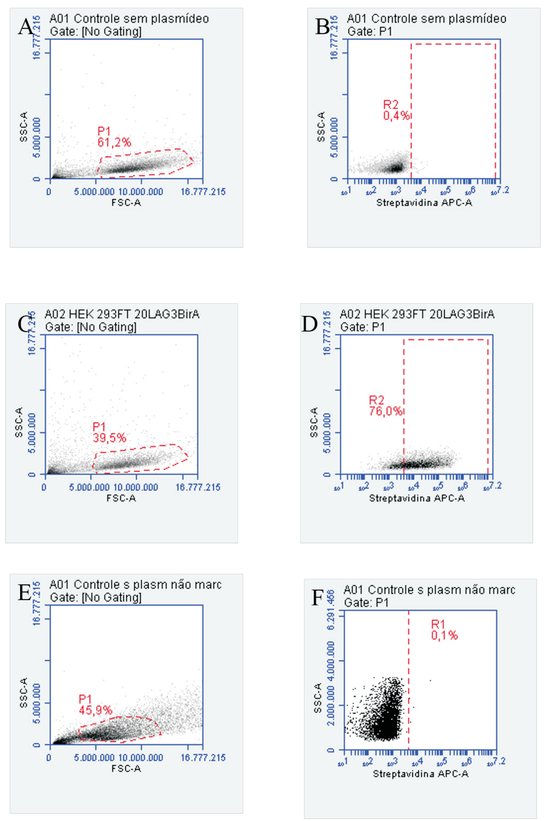




Roux et al., 2012

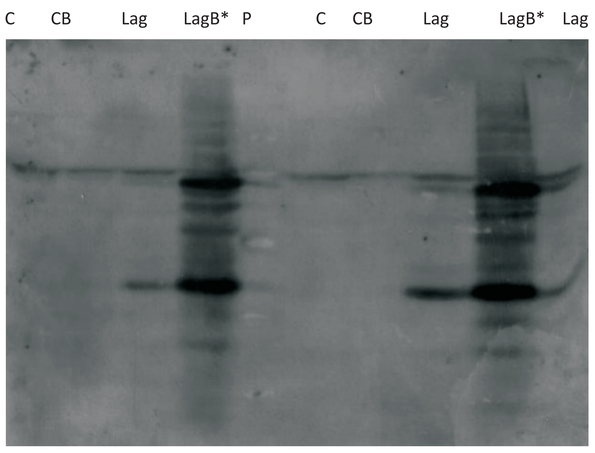
PRELIMINARY RESULTS

Phenotypic characterization of CAR expression (anti-Fab-Fragment antigen binding) by flow cytometry of HEK293FT cell line



Hek FT electroporated cells with 10 µg of 20Lag3 BirA were incubated with anti-Fab antibody (1:200) and anti streptavidin APC (1:200). Cell viability and CAR expression were analyzed after 24h by flow citometry in two independents experiments A) Viable non electropored cells; **B)** Negative control for Streptavidin/APC ; **C)** Viable electroporated cells; **D)** Positive cells for CAR 20Lag3 BirA; **E)** Viable non electropored cells **F)** electroporated cells without anti-Fab incubation ; **G)** Positive cells for anti-Fab-Streptavidina/APC.

Western Blot analysis of biotinylation



Western blot of HEK 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 µM final concentration).

Following SDS-PAGE separation, non transfected and BioID-20Lag3 BirA cells were probed with streptavidin-HRP (Pierce™ High Sensitivity Streptavidin-HRP 1:40.000). **The extensive biotinylation of proteins in the BioID-20Lag3 BirA conditions can be observed.**

(C=Control; CB=Control plus biotin-50µM; Lag=cells electropored with CAR 20Lag3BirA; LagB*= cells electropored with CAR 20Lag3 BirA plus biotin-50µM; P = moleuclar weight marker).

PROSPECTS

- Perform the others electroporations (Lag-3wild type, Lag-3 Kmut (mutation K => Non KIEELE) Lag3 EPdel (EP domain deleted) and Lag-3 Kmut EPdel (double mutant),) in both CARs HEK293FT as in Jurkat 20BBz;
- Recheck the presence of CAR and the standard of biotinylation in all conditions by Westen blot and / or flow cytometry;
- Proceed to the identification and quantification of proteins by mass spectrometry;
- Validate the presence of the proteins identified by flow cytometry and / or Western blot.

CNPq FAPERJ

Projeto Gráfico: Serviço de Edição e Informação Técnico-Científica / INCA

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