OTR.03 - Screening of proteins related to the immunological checkpoint Lymphocyte activation gene-3 (LAG-3) through the BioID method

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Introduction:

Inhibitory receptors such as PD-1, LAG-3, TIM-3 and CTLA-4 have gained special attention as potential targets for immunotherapy, since manipulation of negative signals mediated by these receptors may provide new therapeutic forms for several diseases, as cancer. More recently, Lymphocyte activation gene-3 (LAG-3) was described as a cell surface molecule interacting with MHC class II molecules. Identifying how proteins transduce the signal from these receptors has been a challenge. Once identified, these molecules can also be targets for novel therapeutics. In 2012, in order to identify interactions between proteins in a proximity-dependent manner, Roux et al (2012) created a method called BioID based on the fusion of a protein of interest to a mutated biotin ligase (R118G) (CHOI-RHEE et al., 2004; CRONAN, 2005; ROUX *et al.*, 2012) which has the ability to add biotin to molecules that are at 20 nm or less from the protein of interest. Once biotinylated, the proteins can be recovered by binding to beads conjugated to streptavidin and identified by mass spectrometry.

Objective:

To perform a screening of proteins interacting with LAG-3 through the BioID method as well as to identify the possible signaling pathways modulated by LAG-3 signaling

Methodology:

Chimeric antigen receptors (CARs) were constructed with the anti-CD20 scFv fused to the intracellular domains consisting of: Lag-3 WT, Lag-3 Kmut (K=> A mutation in the KIEELE domain), Lag3 EPdel (deleted EP domain), all fused to the BirA domain, with further induction of expression of these CARs in the HEK293T and Jurkat (CD4+ T lymphocyte) cell lines. Biotin at

a final concentration of 50uM will be added directly to the culture medium and subsequently the cells will be lysed; the proteins recovered through beads conjugated to streptavidin and, after trypsin digestion, analyzed in mass spectrometer. In silico analysis of possible signaling pathways involved downstream to LAG-3 will also be performed based on the labeled proteins identified in mass spectrometry.

Results:

CAR based receptors were synthetized and cloned into pcDNA3.1 vector MCS-BirA (R118G) -HA, using the AgeI and BamHI sites, in frame with the BirA domain. The CAR anti-CD20 / Lag3Wild Type-BirA was electroporated in the HEK293T cell lineage, presenting 80% of expression. CARs Ep del and Kmut were also electroporated into HEK 293T cell lineage showing 39% and 41% of CAR expression. Analysis of the biotinylation pattern by Western blotting was performed (incubation with Pierce ™ High Sensitivity Streptavidin-HRP) for CAR anti-CD20 / Lag3Wild Type-BirA, and the expected ladder pattern of biotinylation was observed.

Conclusion:

The constructed CARs were expressed in the target cell lines leading to the expected biotinilated patterns.

Keywords: LAG3; Chimeric antigen receptor; BioID