GESINAS - ImmunoTools Award 2021

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Reprogramming T cell Glycosylation: a novel immunotherapeutic strategy for Colorectal Cancer

Colorectal cancer (CRC) is the second leading cause of cancer related death worldwide, remaining a serious public health problem in developed regions. Despite the clinical success of immunotherapy, only a minority of CRC patients benefit from this therapeutic modality, highlighting the urgent need for effective CRC therapies.
Changes in glycosylation is a hallmark of cancer. Glycans play a pivotal role in each pathophysiological step of malignant transformation (Pinho&Reis, Nat Rev Cancer 2015) including in cancer immunoediting. Our recent evidences highlight the importance of glycans as relevant immune-checkpoints in cancer immunosurveillance by regulating the cross-talk between tumor and immune cells (Silva&Fernandes *et al.* Cancer Immunol Res 2020). Indeed, the immune system is tightly regulated by glycosylation. Specifically, T cell function is dependent on the glycosylation profile of their cell surface receptors. T cell activity has been shown to be particularly regulated by the expression of β1,6-GlcNAc branched *N*-glycans on the T Cell Receptor, modulating the threshold of T cell activation and signaling (Dias *et al.* PNAS 2018; Pereira&Alves *et al.* Frontiers Immunol 2018). In line with this, we showed that the *ex vivo* metabolic supplementation of mucosal T cells from Ulcerative Colitis patients with *N*-acetylglucosamine led to an enhancement of branched *N*-glycosylation in T cells, associated with suppression of T cell-mediated immune response and disease control (Dias *et al.* PNAS 2018).

Overall, these evidences support the biological relevance of the glycosylation profile of T cells in defining their differentiation, activation and signaling.

Therefore, the main goal of this project is to reprogram T cell glycosignature as a strategy to enhance the effectiveness of immunotherapy for CRC prevention and treatment.

Herein, we will explore the effect of T cells glycoreprogramming by metabolic supplementation with different glycans structures (aiming to promote the biosynthesis of *N*-glycans expression) or using glycosylation inhibitors (such as kifunensine – inhibiting *N*-glycosylation pathway), aiming to evaluate the impact of reprogramming T cell glycosylation in the modulation of T cell activity and effector functions against CRC cells. We will use a murine CRC cell line expressing ovalbumin (MC38-OVA), that will be coculture with both $CD4^+$ T cells from OT-II transgenic mice or $CD8^+$ T cells from OT-I transgenic mice. OT-II-derived $CD4^+$ T cells or OT-I-derived $CD8^+$ T cells will be previously cultured with coated anti-CD3, anti-CD28 and rm IL2 and pre-treated with glycans/ glycosylation inhibitors to glycoengineering T cells. Then, MC38-OVA will be cocultured with these glycoengineered T cells. Human organoids from CRC and precursor lesions will be also established and cocultured with autologous T cells pre-treated with glycans/ glycosylation inhibitors, in the presence of coated anti-CD3, anti-CD28 and rh IL2, as referred above.

To evaluate the effects of T cell glycoreprogramming in the modulation of its activity/function, the cocultures' supernatants will be collected and released cytokines will be analyzed (IFNγ, TNFα, IL10) by ELISA. The impact of presence/absence of specific glycans on T cells differentiation and activity (CD3, CD8, CD4, FoxP3, Tbet, RORgT, Granzyme-B, IFNγ, PD1, CTLA4, CD25, CD69) will be also analyzed by flow cytometry. Additionally, tumor cell death will be analyzed by cytotoxicity assay (Annexin V/Propidium Iodide stain–Flow cytometry).

The ImmunoTools collection of reagents: flow cytometry antibodies, recombinant cytokines and ELISA sets will be essential for the evaluation of the T cell differentiation and activity/function upon glycoreprogramming in the CRC context. With ImmunoTools support, our work will provide new insights on T cell glycoengineering as a novel immunotherapy strategy to enhance anti-tumor immune response in CRC, with potential to have a global impact in the oncology field.

GESINAS – ImmunoTools - Award application:

During the last years, I worked as a volunteer in a national social association "*Associação Moinho de Vermoim*", in the North of Portugal (Famalicão). In this voluntary association, I organized and worked in activities to raise contributions for Portuguese Cancer Foundation "*Liga Portuguesa contra o Cancro*" and "*Médicos sem Fronteiras*". Moreover, I have been involved in activities to communicate science to children, to not only demystify what is a scientist, but also to build, from an early age, bridges between research and the general population.

GESINAS - ImmunoTools AWARD

for **Catarina M. Azevedo** includes 20 reagents

FITC - conjugated anti-human CD3, Annexin-V FITC

PE - conjugated anti-human CD4, CD69, IFN-gamma

PerCP - conjugated anti-human CD8

APC - conjugated anti-human CD3, CD4, CD8, CD25, CD69, Annexin V APC

recombinant human rh IL-2

FITC - conjugated anti-mouse CD8a

PE - conjugated anti-mouse CD3e

APC - conjugated anti-mouse CD4, CD25

recombinant mouse rm IFNgamma, rm IL-2

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