ImmunoTools *special* Award 2018

Athanasios Blanas, PhD student

Supervisor: Dr. Sandra J. van Vliet

Department of Molecular Cell Biology and Immunology (MCBI)
Location VUmc, OI2 building, room 1OE17, De Boelelaan 1108, 1081 HZ Amsterdam, The Netherlands

Impact of altered glycosylation on the tumor growth and the anti-tumor immune response in the context of Colorectal Cancer (CRC)

Project description:
Aberrant glycosylation of tumor cells is recognized as a universal hallmark of cancer pathogenesis, meaning that cancer cells display differential expression levels of critical glycans or distinct carbohydrate epitopes that are not present in the healthy tissue. More specifically, increased levels of fucosylation or sialylation, truncated O-glycans and elevated levels of branched N-glycans on the cell-surface are considered as a well-established signature of malignant cell transformation, identified in many types of cancer including Colorectal Cancer (CRC).

Strikingly, immune cells that are also present within the tumor microenvironment (trying to kill the cancer cells and eventually eliminate the tumor), can sense these tremendous alterations in the glycosylation status of cancer cells through specific glycan-binding proteins that are called C-type lectin receptors (C-type lectins). So far, a number of *in vitro* studies have shown that immune cell-signalling through C-type lectins upon recognition of tumor-associated glycans is often associated with increased secretion of immune-regulatory cytokines (e.g IL-10) and the induction of strong T helper type 2 (Th2) immune response. Therefore, some outstanding research questions still remain to be answered, such as : 1) Do cancer-related glycan structures contribute to the induction of an immunosuppressive tumor microenvironment in the context of CRC? 2) Which carbohydrate motifs and/or C-type lectins instruct these immune-suppression mechanisms exactly? 3) Can novel genome engineering approaches (e.g CRISPR-Cas9) specifically target the glycosylation machinery of cancer cells and reverse this aberrant glycosylation phenotype efficiently?

In order to further dissect the underlying mechanisms of tumor escape from immune surveillance and to successfully address the aforementioned questions, we have exploited the MC38 mouse model, which is a well-known murine CRC model. By knocking-out or transcriptionally reprogramming key glycosyltransferase genes in MC38 cells with the use of the facile CRISPR-Cas9 technology, we have generated a panel of MC38 glyco-engineered cell lines exhibiting differential status of glycosylation on their cell-surface. We are currently injecting MC38 glycovariants

both in the skin (MC38 subcutaneous model) and in the colon (pre-clinical MC38 orthotopic model) of wild-type or C-type lectin-knock out mice, monitoring the tumor growth *in vivo* and assessing the immune cell composition of the progressing tumors *in vitro*. Moreover, we are co-culturing MC38 glycovariants with either Bone-Marrow Dendritic Cells/ Macrophages (BMDCs/ BMDMs) or T cells *in vitro*, focusing on immune cell survival, maturation and cytokine production.

In order to characterize and thus, gain a better insight into the immune cell subsets infiltrating the MC38 tumors, we need detailed antibody panels for subsequent flow cytometric analysis. Therefore, we found several anti-mouse antibodies provided by ImmunoTools (such as the CD3e-FITC, CD80-FITC, Gr-1-FITC, g/d TCR-FITC, isotype control IgG2b-FITC, CD4-PE, CD11b-PE, CD34-PE, CD62-L-PE, a/b TCR-PE, isotype control IgG2b-PE, CD8a-APC, CD44-APC, CD45-APC, Isotype control IgG2b-APC, Annexin-V-APC) that can prove to be extremely useful for our FACS analysis. Also, recombinant mouse cytokines (rm IFN-gamma) and mouse ELISA sets (mouse IL-6, mouse TNF-alpha) can be utilized for our *in vitro* co-culture system approach mentioned above.

In summary, we strongly believe that understanding the effect of aberrant tumor glycosylation on the immune system in the context of CRC can lead to the identification of novel targets for cancer immunotherapy.

ImmunoTools *special* AWARD for **Anthanasios Blanas** includes 25 reagents

APC – conjugated anti-mouse CD8a, CD44, CD45, rat Control IgG2b, Annexin-V

FITC - conjugated anti-mouse CD33, CD80, Gr-1, g/d TCR, rat Control IgG2b

PE - conjugated anti-mouse CD4, CD11b, CD34, CD62L, a/b TCR, rat Control IgG2b

Mouse ELISA-set (for one 96 plate): mouse IL-6, mouse TNF-alpha

recombinant mouse cytokines: rm IFN-gamma

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