Decoding the Crosstalk Between Dendritic Cells and Bladder Cancer Associated Glycans: A Way to Identify Novel Immunotherapy Targets

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Introduction

Bladder cancer (BC) has a complex and multifactorial pathophysiology, contributing to tumour heterogeneity. Due to this, most patients either do not respond to therapy or develop chemoresistance. The appearance of aberrant glycans, namely truncated O-glycans, on tumour cell surface, revealed to contribute to tumour progression (1,2). In particular, truncated O-glycans can exert an immunosuppressive control over immune response against tumours, mainly through engagement with glycan-binding receptors on tumour infiltrated immune cells (3). As such, a more profound understanding of cancer glycome and immune system crosstalk may be key for understanding tumour behaviour and ultimately identifying novel immune checkpoints for cancer immunotherapy (3,4). Given the microenvironmental dependence of glycane signatures on tumour cells, BC cell lines need to be genetically altered to reflect stable glycophnotypes (5).

Aims

1. Development of a cell line library overexpressing glycoconjugates associated with aggressive BC;
2. Validation of the impact of the established cell lines on dendritic cells (DC) maturation;
3. Decoding of the mechanism by which BC-associated glycans are involved in DC modulation.

Methodology

Glycosylation

Glycosylation

Amplicon Analysis (IDAA) and Sanger Sequencing

C1GALT1 KO clone identification

Sanger Sequencing

Phenotype

Model validation

O-glycane characterization by enzyme

Identification of C1GALT1 KO clones by Indel Detection by Amplicon Analysis (IDAA) and Sanger Sequencing

Results

A – Identification of the C1GALT1 KO clones by Indel Detection by Amplicon Analysis (IDAA) and Sanger Sequencing

B – Flow Cytometry for cell surface short-chain O-glycans

C – Immunofluorescence staining for short-chain O-glycan detection in glycoengineered T24 cell line

D – Cellular O-glycome Reporter-Amplification (CORA) method and analysis by mass spectrometry (MS)

Conclusion

According to the data shown, the T24 cell line was successfully genetically edited. As was expected, the C1GALT1 gene KO has induced a simple cell glycoantitype. The KO cell models presented an increased expression of Tn antigen, the appearance of STn antigen, and the inhibition of the formation of core 1 structure and its consequent extension. The analysis of the O-glycan repertoire by MS refined the data obtained by flow cytometry and fluorescence microscopy, confirming the loss of C1GALT1 enzyme activity in the T24 C1GALT1 KO cell models. To increase STn expression, the C1GALT1 KO cell models will be knock-in with the human ST6GALNAc1 gene. The establishment of a well-characterized library of bladder cancer cells, displaying different simple glycoantitypes, will allow for the future crosstalk study between BC-associated glycans and dendritic cells. Such study may enable the identification of promising immunotherapy targets for BC patients faced with limited therapeutic options.

References