GLYCANS AS IMMUNE CHECKPOINTS: REMOVAL OF BRANCHED N-GLYCANS ENHANCES ANTI-TUMOR IMMUNE RESPONSE

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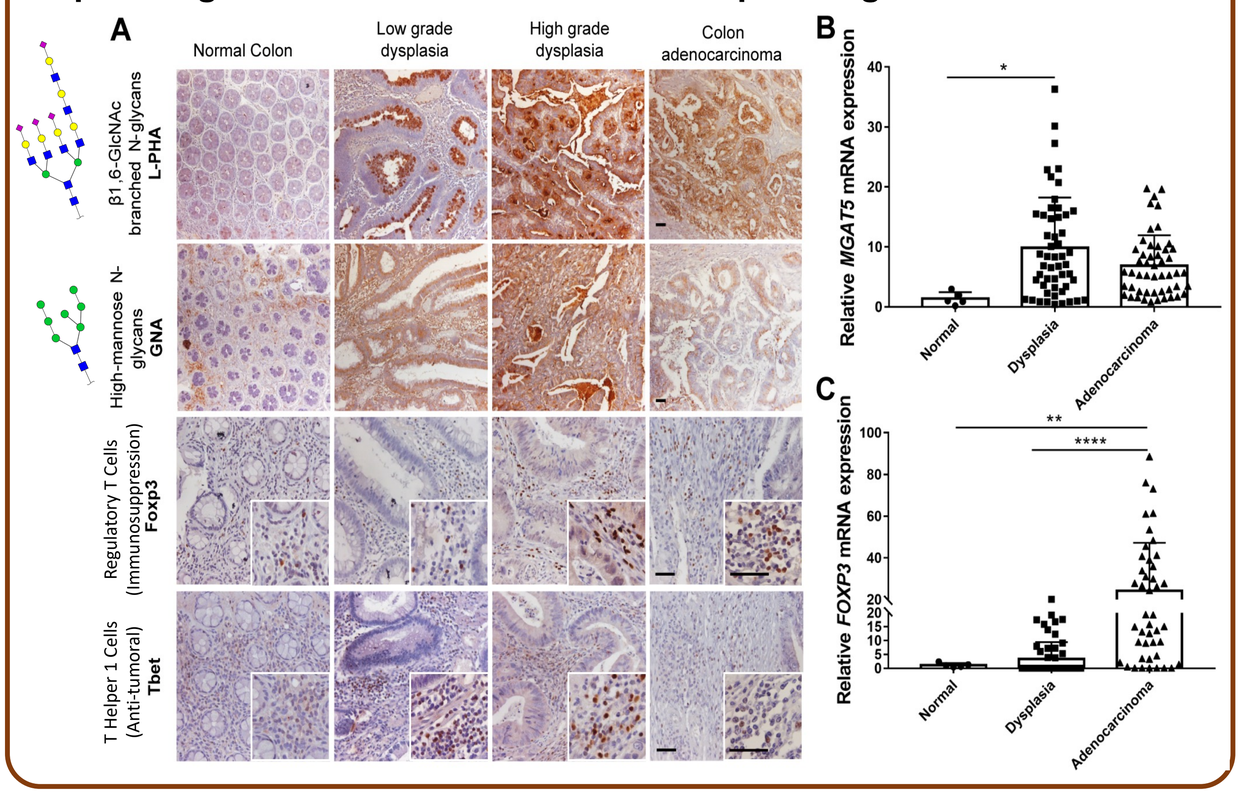
STATE OF THE ART

Colorectal cancer (CRC) is one of the major causes of morbidity and mortality worldwide, being an important target of study. Glycans are carbohydrates that play important roles in several biological processes, including cell-cell and cell-extracellular matrix (ECM) adhesion, receptor activation, molecular trafficking and clearance, endocytosis and signal transduction. Alterations of the glycosylation signature of tumor cells have been shown to be instrumental for the regulation of key mechanisms underlying cancer progression (Pinho & Reis, Nature Rev Cancer 2015). We have been characterizing extensively the tumor glycome, demonstrating that malignant cells overexpress the tumor-associated β1,6-GlcNAc branched N-glycans that have been associated with invasive and metastatic phenotypes and with poor prognosis in gastric cancer patients (Pinho & Reis, Nature Rev Cancer 2015; Carvalho *et al*, Oncogene 2016; Verhelst *et al.* Gastroenterology 2019). Although transformed cells can be recognized by the immune system through immunosurveillance, tumor cells are also able to evade to anti-tumor immune response, through the so-called cancer immune escape process (Dunn *el al.,* Nature Immunology 2002). However, it remains elusive the role of this pro-tumoral N-glycan in immune evasion and whether its removal contributes to enhance immune recognition and to unleash an anti-tumor immune response.

What is the impact of aberrant expression of branched N-glycans in Cancer Immunoediting?

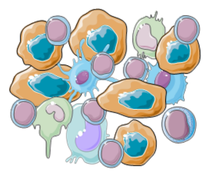
RESULTS

1. Increased expression of β1,6-GlcNAc branched N-glycan along colorectal carcinogenesis is associated with an increase of Foxp3-expressing cells and a decrease of Tbet-expressing cells.



2. The aberrant expression of β1,6-GlcNAc branched N-glycans hampers immune recognition of tumor cells, creating immunosuppressive responses through inhibition of pro-inflammatory cytokine production

MKN45 gastric cancer cell line
- **MKN45 Mock** - MKN45 cells transfected with empty vector
- **MKN45 T5 -** MKN45 cells transfected with *MGAT5* cDNA
  - Overexpressing MGAT5 and consequently, β1,6-GlcNAc branched N-glycans



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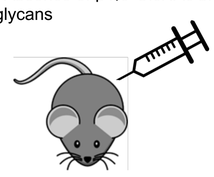
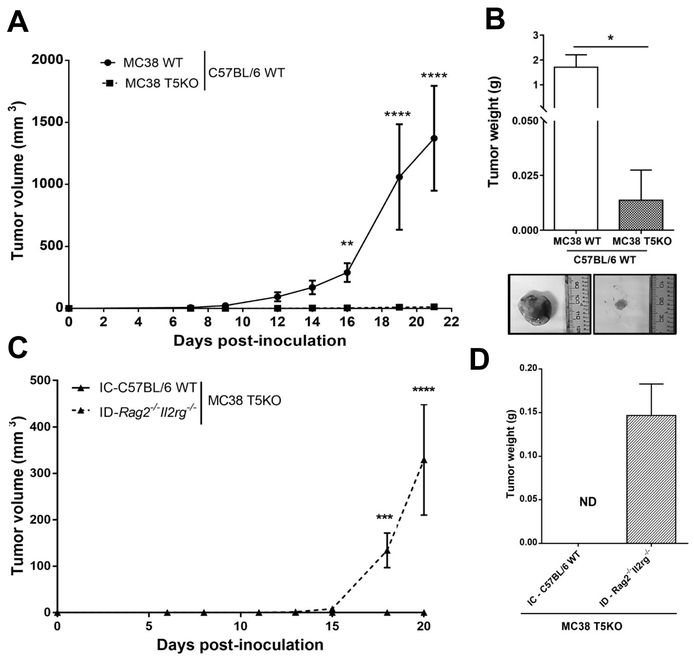
Immune cells (PBMCs) from healthy donors

3. Removing branched N-glycans exposes immunogenic glycans on CRC cells.

MC38 murine colorectal cancer cell line

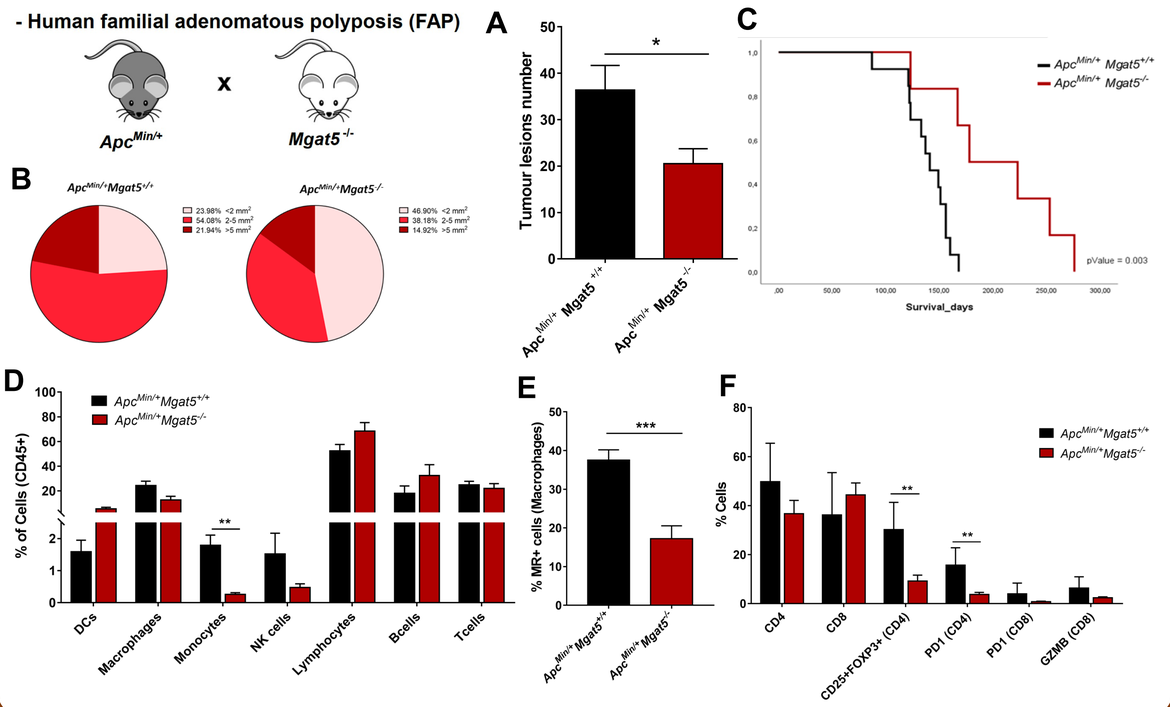
- **MC38 T5KO -** MC38 cells knockout to *Mgat5* gene (CRISPR/Cas9)
  - Absence of β1,6-GlcNAc branched N-glycans





4. In vivo deletion of branched glycosylation results in spontaneous suppression of CRC through unleashing an anti-tumor immune attack

- Human familial adenomatous polyposis (FAP)



CONCLUSION

β1,6-GlcNAc branched N-glycans ↑

↓ **Pro-inflammatory cytokine** production
IL6 and **IL8**

↓ **Tbet** transcription factor expression

↓ **IFNγ** and **TNFα**-producing CD4 T cells

Th1-type CD4 T cells

✖ **β1,6-GlcNAc branched N-glycans**

↑ **Immunosurveillance**

↓ **Number** and **size** of tumors

Potential new mechanism of **Cancer Immunoediting in Colorectal Cancer**

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