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22860 | Deciphering the Influence of CD276 glycocode in Gastric Cancer Immunoregulation

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Background & Aim: Gastric cancer (GC) ranks 5th globally in incidence and cancer-related mortality, with advanced-stage 5-year survival rates below 20%[1,2], unveiling the need for novel immunotherapies. CD276, an immune checkpoint molecule, is related to immune evasion and tumour progression in GC[3-5]. In turn, aberrant glycosylation, especially truncated *O*-glycans, Tn and Sialyl-Tn, correlates with GC aggressiveness and poor prognosis[6]. Its clinical impact relies on the modulation of protein function and immune escape[5,7]. This study hypothesizes that CD276 glycosylation may offer new targets for immunotherapy in GC. **Methods:** CD276 expression was assessed in AGS and MKN-45 cell lines using qPCR and immunoblotting. Glycoengineered AGS and MKN-45 cell lines with truncated glycosylation (C1GALT1 KO) and wild-type (WT) controls were analysed similarly. *In vitro* proliferation, migration and invasion assays were conducted. **Results:** Both cell lines have CD276 expression in WT cells. CRISPR/Cas9-mediated knockout of C1GALT1 did not significantly alter CD276 protein or mRNA expression levels. The proliferation assay did not show any changes in either cell line. However, migratory and invasive assays promoted a cell line-dependent response. The migratory capacity of AGS decreased with truncated glycosylation while increasing for MKN-45. C1GALT1 KO AGS cells showed enhanced invasiveness. **Conclusion:** Aberrant glycosylation in AGS and MKN-45 cells did not affect CD276 expression *in vitro*, suggesting a CD276 expression independent of *O*-glycan elongation in GC. Tn/STn glycophenotypes have a cell line-dependent functional impact in GC, suggesting glycosylation-related migration and invasion capacities. Future proteomic studies will clarify the results. Moreover, further research will identify CD276 glycoproteoforms and assess CD276 abrogation effects on T-cell immunomodulation and cytokine profiles. This research may

contribute to the development of novel targeted therapies and improved patient outcomes in GC.

Keywords: Gastric cancer, *O*-glycosylation, CD276.

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