EFFECT OF *N*-GLYCAN BRANCHING DEGREE ON THE LEVELS OF UDP-GLCNAC, O-GLCNAC AND HYALURONIC ACID IN COLORECTAL CANCER CELLS

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ABSTRACT

Introduction: Tumor cells, including CRC, undergo metabolic reprogramming characterized by several changes in energetic metabolism, such as high glucose uptake rate and the "Warburg effect" (aerobic glycolysis). Glycolytic pathway intermediates are used to supply anabolic processes, such as the hexosamine biosynthesis pathway (HBP) or the pentoses pathway. In fact, ~2.5 percent of a cell's glucose is directed to HPB and its final product (the sugar donor nucleotide UDP-GlcNAc) is crucial to many glycans and glycosaminoglycans biosynthesis. An important question about cancer biology is how changes in glycans expression profile are related to carcinoma progression, because in many cases it is still unclear. Studies have shown that β1,6-GlcNAC branched N-glycans are associated with acquisition of migratory phenotype and loss of the apical junctional complex stability. In tumor cells, changes in the levels of both hyaluronic acid and O-GlcNAc (both dependent on UDP-GlcNAc) have been reported to contribute with acquisition of malignant phenotype. Thus, the aim of this project is to evaluate, in colorectal cancer cells, the effect of N-glycan branching degree on the levels of UDPGlcNAc, O-GlcNAc and hyaluronic acid, under high and low glucose conditions, with the purpose of verifying the existence of a integrated metabolic-structural mechanism involved in multiple characteristics associated to the malignant phenotype.

PROPOSED MODEL

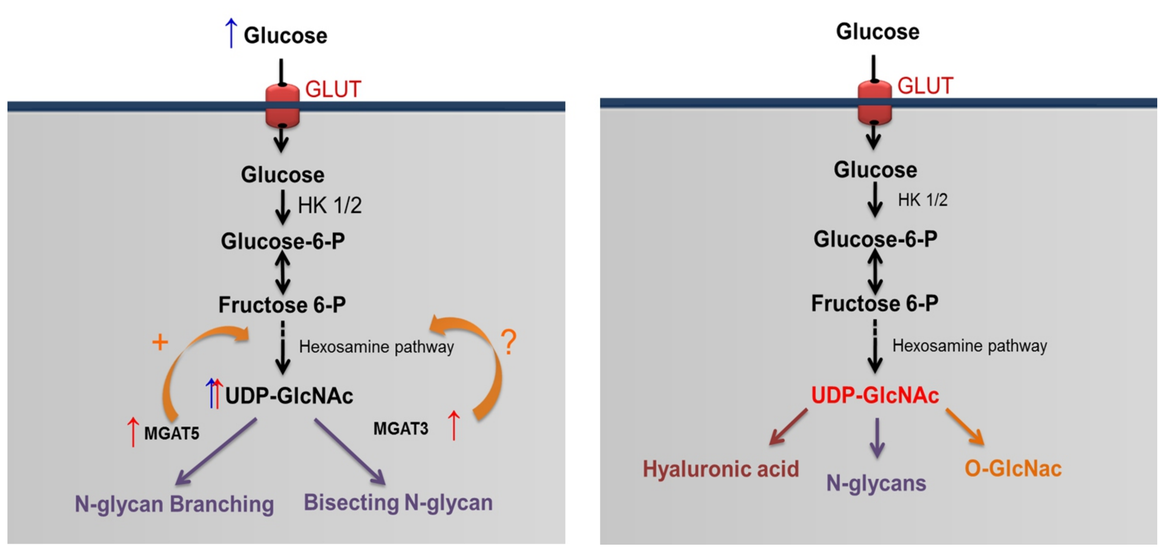

Figure 1: Scheme that summarizes the rationale proposed in this study project. The influence of increased or decreased glucose uptake and hexosamine pathway, and consequent increase in UDP-GlcNAc production, may modulate the expression of glycosyltransferases such as MGAT5 and MGAT3, which may influence the production of branched and bisected *N*-glycans, respectively. The effect of *N*-glycan branching degree on the levels of UDP-GlcNAc, *O*-GlcNAc and hyaluronic acid, under high and low glucose conditions, will be evaluated in this project, aining to identify the existence of a integrated metabolic-structural mechanism involved in multiple characteristics associated to the malignant phenotype.

METHODS AND RESULTS

CRC cell line were cultured under high or low glucose conditions and a flow cytometry was developed using specific lectins to investigate changes in branched N-glycan levels, as well as other glycan structures levels. Before 24h swainsonine treatment (an inhibitor of complex N-glycan biosynthesis, which prevent the formation of branched structures), we investigate the variation of UDP-GlcNAc levels, through HPLC method. Changes in the proliferation and expression of E-caderin profile was observed in different glucose conditions. Next, for this project, the protein post-translational modification with O-GlcNAc will be evaluated by Western blotting, and hyaluronic acid will be indirect measured by HAS (Homo sapiens hyaluronan synthase) gene in CRC cells cultured different glucose conditions and treated with N-glycan biosynthesis inhibitors.

PRELIMINARY RESULTS

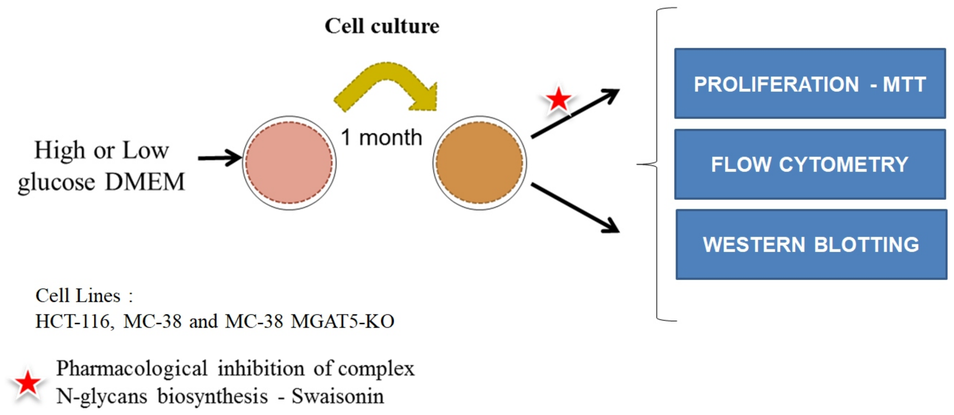

Figure 2: Experimental strategy. The cell line will be maintained for 1 month in high or low glucose DMEM medium for metabolic reprogramming, followed by pharmacological inhibition of complex *N*-glycans biosynthesis by Swaisonine. Subsequently, High glucose (HG) or Low glucose (LG) with or without treatment with swaisonine will be evaluated by the assays described above.

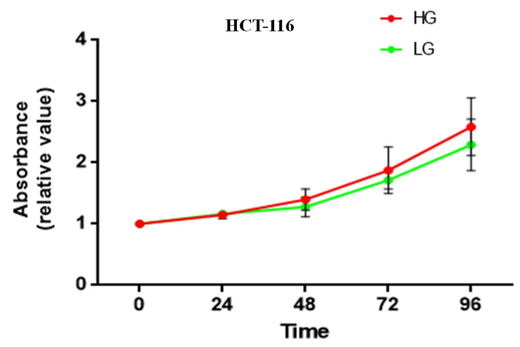

Figure 3: No significant change was observed in 96-hour cell growth profile in HCT-116 cells cultured under different glucose concentrations. HCT-116 cells were cultured in high (HG) or low glucose (LG) DMEM medium for 1 month, and the proliferative effect was analysed by MTT assay after 24, 48, 72 and 96 hours. The results are expressed as mean ± s.e.m. of three independent experiments.

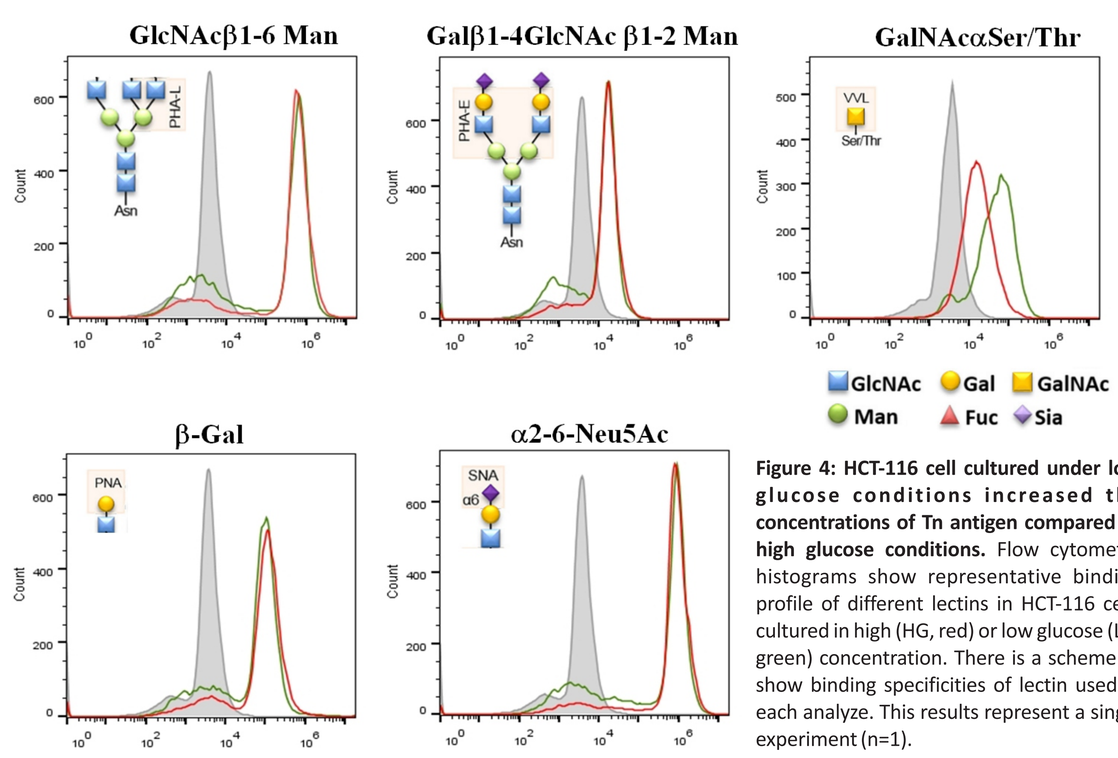

Figure 4: HCT-116 cell cultured under low glucose conditions increased the concentrations of Tn antigen compared to high glucose conditions. Flow cytometry histograms show representative binding profile of different lectins in HCT-116 cells cultured in high (HG, red) or low glucose (LG, green) concentration. There is a scheme to show binding specificities of lectin used in each analyze. This results represent a single experiment (n=1).

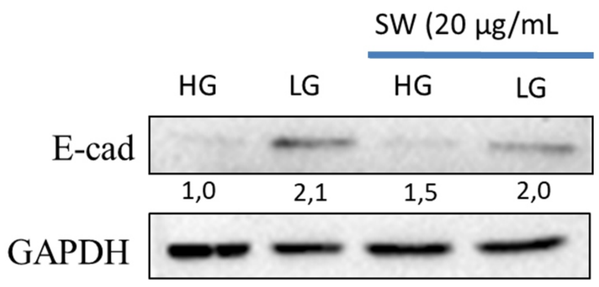

Figure 5: Increased levels of E-cadherin induced by low glucose conditions and Swaisonine treatment in HCT-116 cells. HCT-116 cells (HG and LG) were treatated with 20 µg/mL an inhibitor of complex N-glycan biosynthesis, swaisonine (SW), and analyzed by immunoblotting assay. The E-cadherin levels in lysates were analyzed after 24h of tratment. The results are expressed as mean of two independent experiments. *Numbers below the figure* represent the ratio of the optical density of the bands as fold change of protein expression normalysed by GAPDH.

CONCLUSION

Taking into account these metabolic-structural mechanisms, we believe the present work will may contribute to a better understanding of the CRC development and progression.

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