ROLE OF INSR/IGF1R SIGNALING IN REGULATING N-GLYCANS AND MALIGNANT PHENOTYPE OF COLORECTAL CANCER CELLS

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ABSTRACT

Colorectal cancer (CRC) is a leading cause of cancer-related mortality. During CRC progression several genetic and molecular changes affect the functionality of signaling-related proteins, including those that are part of the insulin/insulin-like growth factor (IGF) system. In addition, metabolic changes that culminate in aberrant glycosylation are also considered to be a hallmark of cancer, because evidence has shown that glycans can modulate cell migration, cell-cell adhesion, cell signaling, and metastasis. Although several studies have contributed to a better understanding of the physiological and pathological molecular mechanisms mediated by insulin/IGF system and glycans independently, an integrated approach linking this system to glycosylation changes in CRC development remains lacking. The aim of this study is to understand how disturbances in the insulin/IGF system affect both malignant phenotype and N-glycan expression pattern, mainly concerning β1,6-GlcNAc-branched N-glycans, which are products of the N-acetylglucosaminyltransferase V (MGAT5), known to be involved in destabilization of adherens junctions (AJ) and acquisition of migratory phenotype. For this purpose, OSI-906 - a potent and highly selective inhibitor of tyrosine kinase molecules that inhibit insulin receptor (INSR) and IGF1 (IGF1R) autophosphorylation - was used for the treatment of HCT-116 and HT-29 cells. In both cases, OSI-906 lead to a dose-dependent significant decrease in cell viability. Moreover, inhibition of the insulin/IGF pathway has been seen to affect the levels of AJ-related proteins, because the treatment with OSI-906 promote both a decrease in β-catenin and an increase in E-cadherin levels, associated with a change in HCT-116 cell shape, which acquire a higher level of cell compaction. Also, in this cell line, two treatment strategies were maintained, one with OSI-906 alone, and another with IGF prior to treatment with the inhibitor. Under both conditions, there was a decrease in β1,6-GlcNAc-branched N-glycans (L-PHA positive), which was also observed in HT-29 cells treated only with OSI-906. Together, these preliminary findings indicate a correlation between INSR/IGF1R signaling and the levels of β1,6-GlcNAc branched N-glycans.

INTRODUCTION

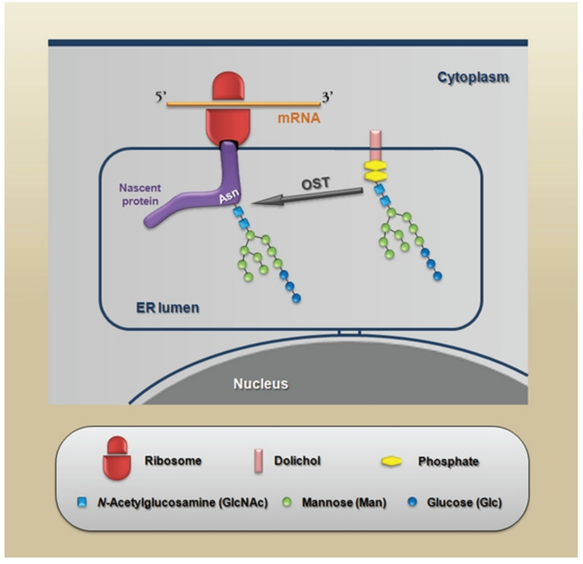

Figure 1: Schematic representation of the protein *N*-glycosylation reaction. The nascent proteins synthesized within the endoplasmic reticulum are glycosylated via the *en bloc* transfer of a precursor oligosaccharide to an asparagine residue in a specific sequence (Asn-X-Ser / Thr, where X is any amino acid except proline). This reaction is catalyzed by OST (oligosaccharyltransferase).

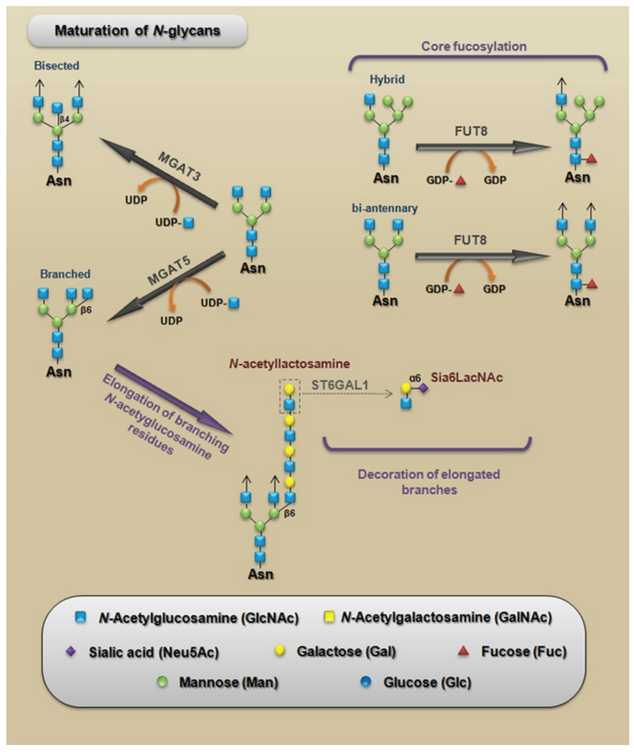

Figure 2: Maturation of N-glycans. A schematic representation shows the three components of maturation N-glycan: (I) Core fucosylation; (II) extension of branches formed by N-acetylglicosamines; and (III) decoration of the extensions (antennas). It is important to note that the branched N-glycans formed by β1,6GlcNAc and the β1,4 GlcNAc bisected are, respectively, MGAT3 (N-acetylglicosamyltransferase III and MGAT5 (N-acetylglicosamyltransferase V) products. Changes in the expression patterns of these N-glycans have been associated with several pathologies, including carcinomas.

PROPOSED MODEL

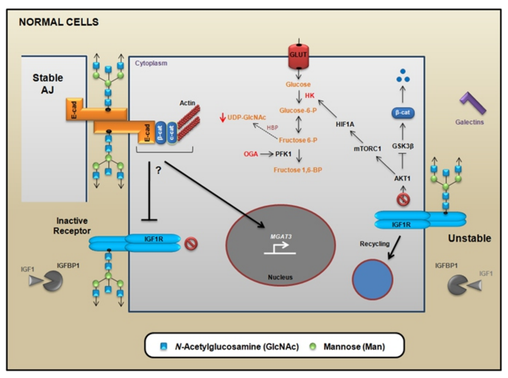

Figure 3: Signaling/metabolic network under low degree of *N*-glycan branching in normal cells. The stability of adherens junctions is favored by expression of the MGAT3 products, thus establishing a reciprocal mechanism via positive regulation of MGAT3 expression. In addition, inactivation of IGF1R when stabilized by β1,4-GlcNAc bisected *N*-glycans, theres is an inhibition of the AKT1 pathway, subsequently, stimulate GSK3β and there is a down-regulation of HK, a reduced pathway of the hexosamines (HBP), thus decreasing a biosynthesis of GlcNAc.

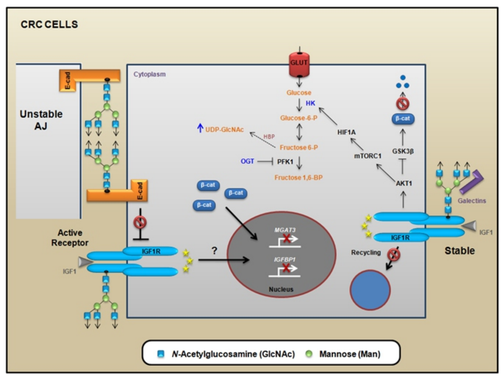

Figure 4: Signaling/metabolic network under high degree of N-glycan branching in CRC cells. High degree of *N*-glycan branching on the one hand destabilizes E-cadherin-mediated cell-cell adhesion and, on the other hand, favors the stabilization of IGF1R due to its interaction with galectins, thus increasing both AKT1/mTORC1/ HIF1A/HK axis and inhibitory effects on GSK3β mediated by AKT1. As a consequence of the high HK activity, the HBP flux become higher, increasing the GlcNAc biosynthesis. Concomitantly to repression of IGFBP1 the translocation of cytoplasmic β-catenin to nucleus promotes inhibition of MGAT3 expression, thus establishing a positive feedback mechanism on IGFR1 activity. synthesis of GlcNAc.

EXPERIMENTAL STRATEGY

In order to carry out the experiments, the following cell lines derived from human colorectal cancer were maintained in culture: HCT-116 (ATCC catalog CCL-247), RAS mutated, undifferentiated, and invasive and; HT-29 (HTB-38 catalog), poorly differentiated, highly invasive and mutated in P53 and RAS. Cell viability analysis was performed using MTT, while the efficiency of inhibition of INSR and IGF1R by the OSI-906 compound has been monitored by assessing the state of phosphorylation of receptors by immunoblotting. Moreover, the expression levels of bisected and branched N-glycans have been monitored by flow cytometry and lectin blotting using the E-PHA lectin (specific for bisected N-glycan with β1,4GlcNAc) and L-PHA (specific for tri- and tetra-antennary complex-type N-glycans with β1,6-linked N-acetylglucosamine).

PRELIMINARY RESULTS

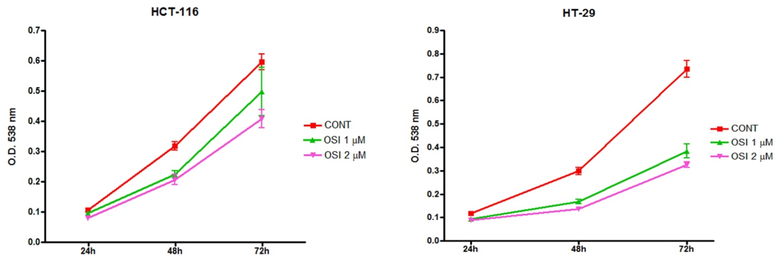

Figure 5: Treatment with OSI-906 for 48h lead to a decrease in cell viability in HCT-116 and HT-29. Cells were plated and cultured in a 96-well plate. Cell viability was analyzed by the MTT assay at 24h, 48h and 72h

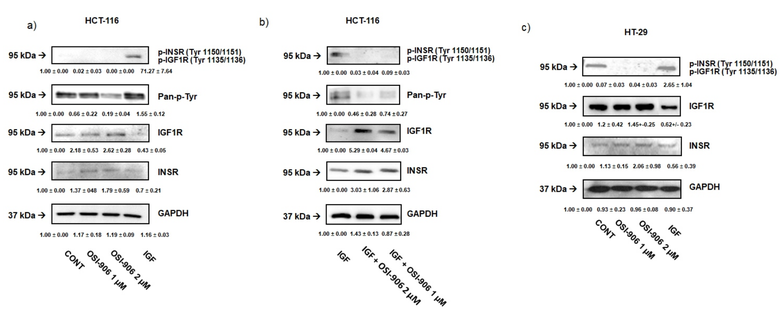

Figure 6: Efficacy of OSI-906 in HCT-116 and HT-29 cells. After the 48 hour of treatment, Western blotting was performed to analyze the level of phosphorylation of the protein (p-IRIGF-1R) in the cells cited

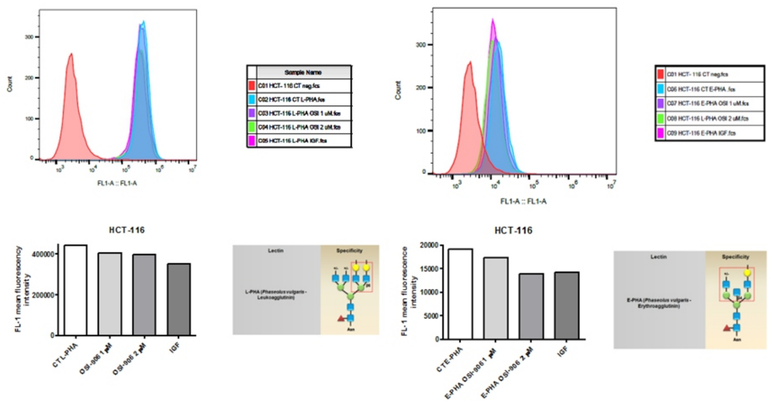

Figure 7: Effects of repression of INSR / IGF-1R mediated signaling on N-glycan levels in HCT-116 cell. There was a decrease in both the branched (left panel) and the bisected (right panel)

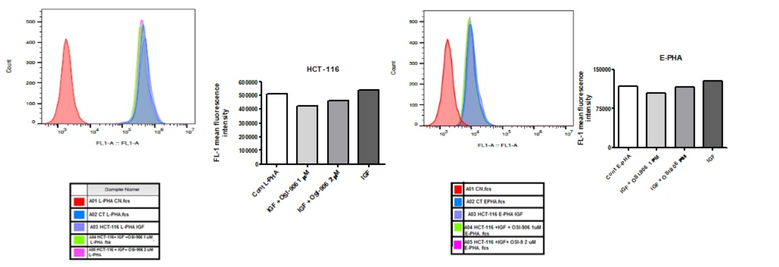

Figure 8: Effects of repression of INSR / IGF-1R-mediated signaling on N-glycan levels in HCT-116 cells. Such a similar pattern was observed in cells that were pretreated with IGF, with the decrease in N-glycans positive for L-PHA (left panel) and for those positive E-PHA (right panel)

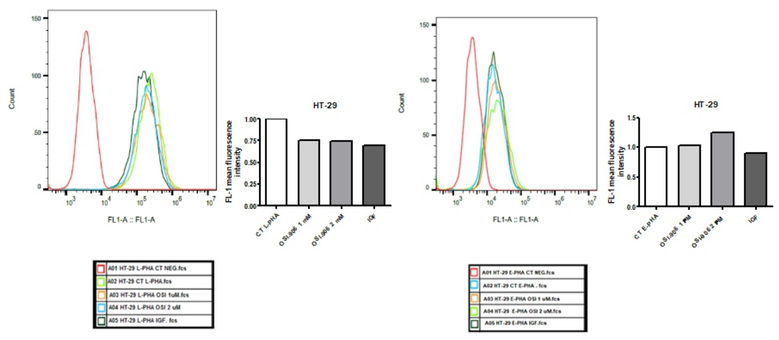

Figure 9: Effects of repression of INSR / IGF-1R mediated signaling on *N*-glycan levels in HT-29 cell. A decrease in N-glycans positive for L-PHA (left panel) was observed, while an increase in *N*-glycans positive for E-PHA (right panel)

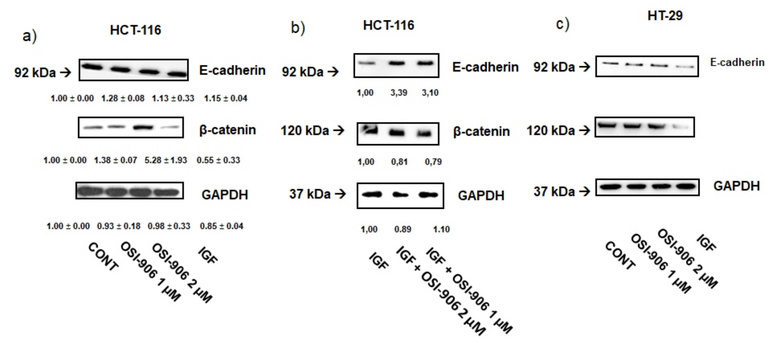

Figure 10: Inhibition of insulin / IGF pathway affects levels of junction proteins in HCT-116 and HT-29 cells. In cells treated with OSI-906 alone, E-cadherin levels remained similar to control, however, -catenin showed an increase, especially at 2 μM (a). In HT-116 cells where prior treatment with IGF occurred, inhibition by OSI-906 promoted an increase in E-cadherin expression and a decrease in -catenin levels (b) ; In HT-29, there was an increase in E-cadherin expression in both concentrations of OSI-906, whereas -catenin, similar to control, however, when stimulated by the agonist, showed a decrease.

CONCLUSION

Together, these preliminary findings indicate a correlation between INSR/IGF1R signaling and the levels of β1,6-GlcNAc branched *N*-glycans.

PERSPECTIVES

After performing new replicates to confirm the results obtained so far, the functional tests will be started. Migration and invasion analyzes will be performed respectively by transwell and transwell coated with matrigel. While the integrity of the adherent junctions will be evaluated by immunofluorescence and transmission electron microscopy. Monitoring of glucose uptake rates will be performed by colorimetric assay

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