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 mammals this system comprises a dynamic network of proteins that modulates several biological processes, such as development, cell growth, metabolism, and aging. Dysregulation of insulin/IGF system is implicated in both diabetes and cancer development. 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*-glycans 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. For this purpose, will be evaluated the effects of OSI-906 – a potent and highly selective small molecule tyrosine kinase inhibitor which inhibits autophosphorylation of both IGF1 and insulin receptors (IGF1R and INSR) – on the levels of: β1,6 branched *N*-glycans; MGAT3 (enzyme whose product are β1,4-GlcNAc bisected *N*-Glycans that cannot be used as a substrate by MGAT5); and IGFBPs (proteins that modulate IGF1 bioavailability). Moreover, will be also evaluated the effects of OSI-906 on the glucose uptake, migratory and invasive potential, and adherens junctions integrity.

INTRODUCTION

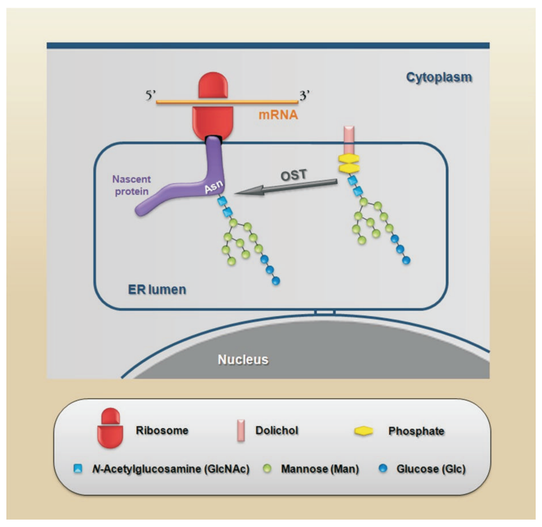

Figure 1: Schematic representation of the protein N-glycosylation reaction. The nascent proteins synthesized within the endoplasmic reticulum are glycosylated via the *en bloc* transfer of a precursor oligosaccharide to an asparagine residue in a specific sequence (Asn-X-Ser / Thr, where X is any amino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

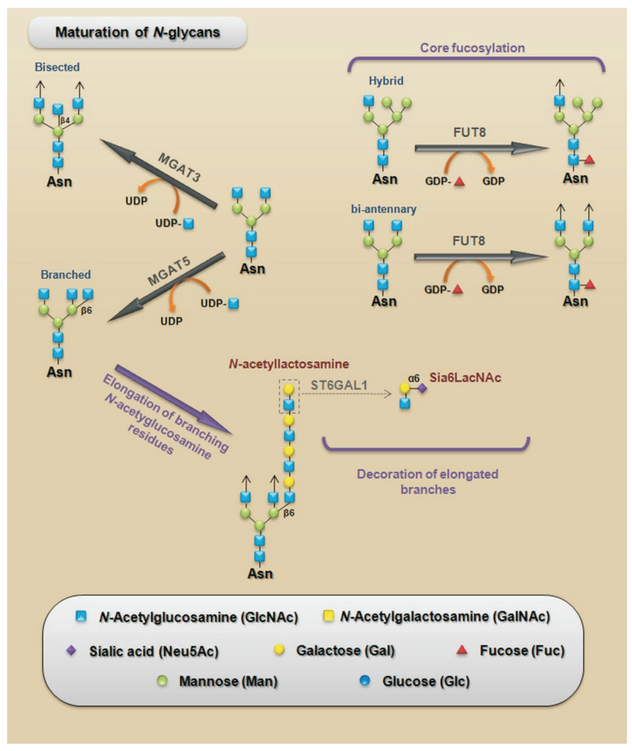

Figure 2: Maturation of N-glycans. A schematic representation shows the three components of maturation N-glycan: (I) Core fucosylation; (II) extension of branches formed by N-acetylglicosamines; and (III) decoration of the extensions (antennas). It is important to note that the branched N-glycans formed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

EXPERIMENTAL STRATEGY

In order to carry out the experiments the HCT-116 cell line (derived from undifferentiated human colon adenocarcinoma, ATCC catalog CCL-247) has been routinely maintained in culture. 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. The expression levels of bisected and branched *N*-glycans have been monitored by flow cytometry and lectin blotting using the specific E-PHA lectin (*N*-glycan bisected with β1,4GlcNAc branches) and L-PHA (branched tri- and tetra-antennary complex-type *N*-glycans with β1,6-linked *N*-acetylglucosamine).

PROPOSED MODEL

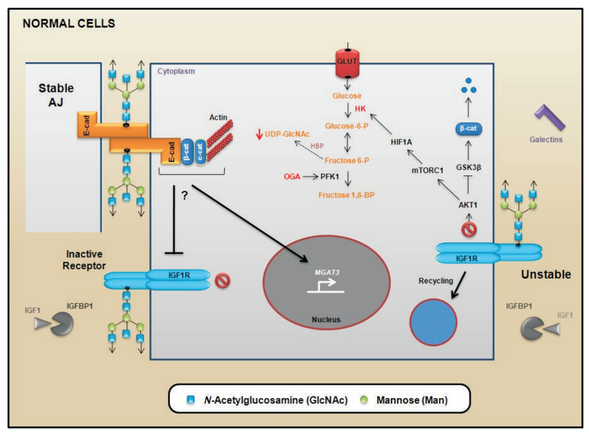

Figure 3: Signaling/metabolic network under low degree of N-glycan branching in normal cells. The stability of adherens junctions is favored by expression of the MGAT3 products, thus establishing a reciprocal mechanism via positive regulation of MGAT3 expression. In addition, inactivation of IGF1R when 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.

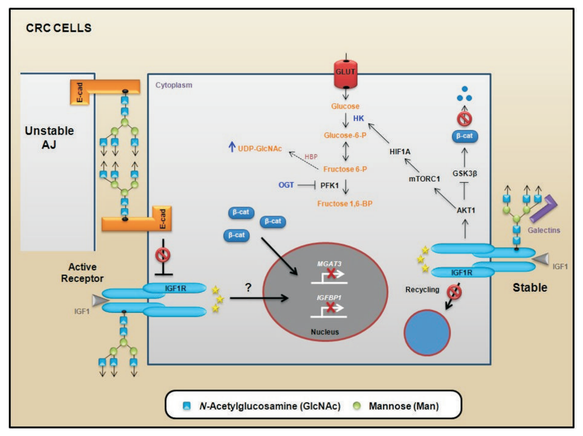

Figure 4: Signaling/metabolic network under high degree of *N*-glycan branching in CRC cells. High degree of N-glycan branching on the one hand destabilizes E-cadherin-mediated cell-cell adhesion and, on the other hand, favors the stabilization of IGF1R due to its interaction with galectins, thus increasing 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.

PRELIMINARY RESULTS

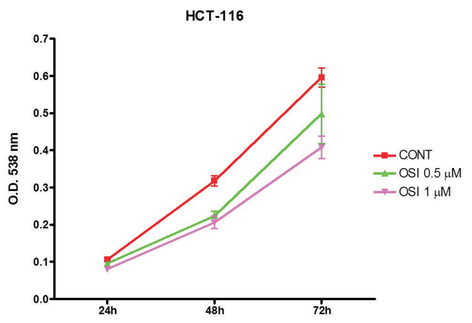

Figure 5: Treatment with OSI-906 for 48h promotes decreased viability in HCT-116 cells. Cells were plated and cultured in a 96-well plate. Cell viability was analyzed by the MTT assay at 24h, 48h and 72h times.

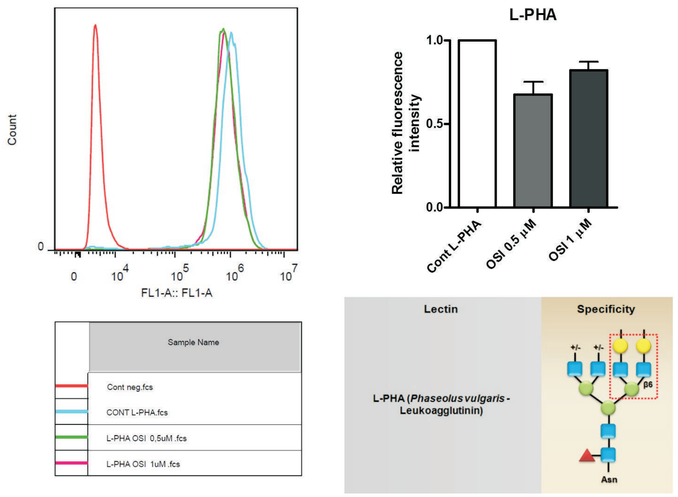

Figure 6: Treatment with OSI-906 reduces branched N-glycans expression levels of of in HCT-116 cells. After 48 hours of treatment, the cells were trypsinized and, through quantification the fluorescence intensity by flow cytometry, a decrease in L-PHA-positive N-glycan levels as compared to the control was observed.

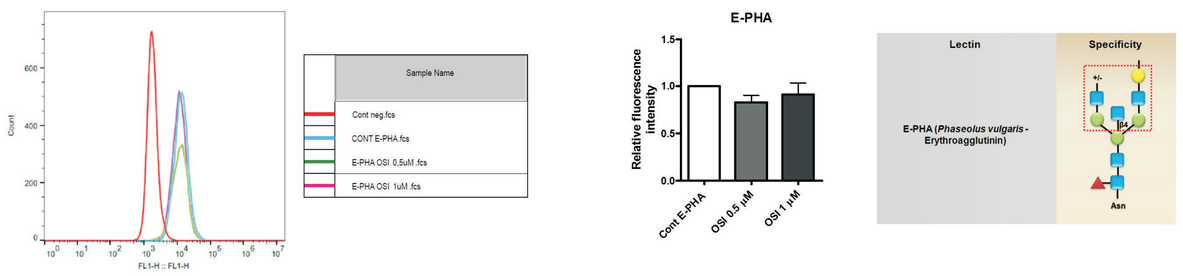

Figure 7: OSI-906 treatment and expression levels of bisected N-glycans in HCT-116 cells. After 48 hours of treatment, the cells were trypsinized and, through quantification the fluorescence intensity by flow cytometry, no difference in their expression levels were detected .

RESULTS(PRELIMINARY)

After performing new replicates to confirm the results obtained so far, the functional tests will be started. Cell viability, migration and invasion analyzes will be performed respectively by MTT, 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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