Influence of the Hexosamine Biosynthetic Pathway on Metabolic Dysregulation Linked to ?1,6-GlcNAc-Branched N-Glycans in colorectal cancer progression

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Introduction: Malignant tumors often exhibit significant metabolic alterations, including increased glucose (Glc) and amino acid (e.g., glutamine - Gln) consumption. Another common change is the exacerbation of the hexosamine biosynthetic pathway (HBP), a glycolysis branch that produces the nucleotide sugar UDP-*N*-acetylglucosamine (UDP-GlcNAc). Glycan structures dependent on UDP-GlcNAc, such as branched *N*-glycans, *O*-GlcNAc in cytosolic and nuclear proteins (OGP), and hyaluronic acid (HA), are linked to tumorigenic processes and metastasis. **Aim:** This study investigated the influence of the HBP on metabolic dysregulation related to glycosylation processes utilizing UDP-GlcNAc, with a focus on the role of MGAT5-mediated synthesis of branched *N* -glycans in colorectal cancer (CRC). **Methods:** We used metabolomics analyses to assess the impact on cell metabolism, specifically intermediates of the HBP and UDP-GlcNAc production, following the inhibition of ?1,6-GlcNAc-branched *N*-glycan synthesis through *Mgat5* knockout (KO *Mgat5*). Microarray analysis and functional profiling (including proliferation, clonogenic, migration, and invasion assays) were performed on KO *Mgat5* cells. To determine which processes—?1,6-GlcNAc-branched *N*-glycan, *O*-GlcNAc, and HA synthesis—are most affected by UDP-GlcNAc restriction, we inhibited GFAT, a key HBP enzyme. To assess the effects of impaired branched *N*-glycan synthesis and GFAT inhibition on Glc or Gln influx, we quantified the uptake of these molecules. Additionally, TCGA data were used to correlations analyses regarding *MGAT5* and/or *GFPT1* and *GFPT2* (GFAT genes) expression and key genes in the HBP, Glc/Gln transport, and glycan synthesis pathways, and their relation to CRC progression. **Results:** Metabolomics analyses revealed that inhibiting ?1,6-GlcNAc-branched *N*-glycan synthesis negatively impacts intermediates of the HBP, including UDP-GlcNAc, as well as several other activated monosaccharides. These alterations led to the formation of two distinct groups of metabolically different cells, with ?-ketoglutarate, an intermediate of glutamine metabolism, being one of the most important discriminant metabolites responsible for this metabolic separation. Additionally, we observed a decrease in Gln consumption by cells in the absence of ?1,6-GlcNAc-branched *N*-glycans. Potential reductions in OGP and HA synthesis were also observed. *Mgat5* knockout cells exhibited reduced proliferative, colony-forming, migration, and invasion capacities. Moreover, UDP-GlcNAc restriction caused by GFAT inhibition resulted in a significant impairment of OGP levels, with no changes in HA levels. Interestingly, we also observed increased levels of branched *N*-glycans, along with an increased Gln consumption. Finally, we found that *MGAT5*, *GFPT1*, and *GFPT2* expression levels are distinctly related to CRC survival, demonstrating MGAT5 as a potential prognostic marker, given the association of its increased expression with poorer disease-free survival. **Conclusion:** Together, our results suggest an interrelationship between different demands for UDP-GlcNAc in tumor cells, contributing to a better understanding of the interconnection between biochemical processes that require UDP-GlcNAc in the context of colorectal cancer.

Agradecimentos: