Unraveling mucin type o-glycosylation signatures of colorectal cancer

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ENGLISH SUMMARY

The surface of eukaryotic cells contains a very dense layer of oligosaccharides called glycans that are linked to protein and lipid carriers and play an important role in cell-cell and cell-extracellular matrix interactions. Aberrant glycosylation is a hallmark of cancer and plays a role in various cellular processes that are involved in cancer pathogenesis, such as cell proliferation, adhesion, migration and immune surveillance. Cancer-induced changes in glycosylation have an impact on the function of major glycoproteins in the human colon, therefore studies focused on colorectal cancer (CRC)-specific glycosylation signatures can provide novel insights into onset and progression of this disease. The major focus of this thesis was to investigate mucin type *O*-glycosylation signatures of CRC. For this purpose, a protocol for in-depth analysis of *N*- and *O*-glycans obtained from cell lines was developed (**Chapter 2**) using nanoscale porous graphitized carbon liquid chromatography coupled to mass spectrometry (PGC-nano-LC-MS). The sample preparation protocol was adapted to a robust 96-well plate format to increase throughput of release and purification compared to previous cell line approaches. This strategy, furthermore, allowed glycan isomer separation and detailed structural identifications. In **Chapter 3** additional conditions were optimized in the MS methodology by using polar protic dopant (methanol and isopropanol) enriched nitrogen gas to increase sensitivity on the MS and tandem MS level. This allowed for a more confident structural identification as well as correction for the ionization bias for early eluting species in the LC-gradient. In **Chapter 4** we applied the methodology developed in Chapter 2 to the analysis of *O*-glycosylation signatures of 26 different CRC cell lines. This analysis resulted in the characterization of more than 150 O-glycan structures and increased our understanding of glycan expression in the analyzed cell lines. The glycophenotypes showed correlation mainly to cell differentiation. Namely, colon-like well-differentiated cell lines expressed Lewis type antigens and I-branched glycans, whereas undifferentiated cell lines expressed sialylated core 1 glycans and partially blood group H-antigens. From pathway analysis it was determined that differences in expression of the glycosyltransferases and transcription factors in those groups associated with the glycan signatures. This revealed that these changes reflect the differences in the glycosylation machinery, and not per se the abundances of specific proteins. To gain further understanding in the mechanisms underlying glycomic changes with colon cell

differentiation, we explored changes in the cell line glycome and proteome upon spontaneous and butyrate-stimulated differentiation in in vitro cell culture (**Chapter 5**). Glycomic analysis revealed a decrease of blood group antigen H fucosylation with cellular differentiation, together with increased sialylation levels. Increased expression of specific cell adhesion proteins was found on the proteome level. By performing an integrative approach, we generated hypotheses about glycosylation signatures of specific cell adhesion proteins, which may play an important role in cancer progression. The localization of glycans on the cell surface and their role in biological processes are important in cancer pathogenesis, making them potential candidates for glycan targeting immunotherapy. Therefore, we further optimized the methodology to enable comprehensive analysis of *N*- and *O*-glycans from specific regions of formalin-fixed, paraffin-embedded tissues using laser capture microdissections and applied it for the analysis of selected regions of CRC tissues and their patient-matched colon mucosa controls (**Chapter 6**). We identified specific tumor-associated carbohydrate antigens (TACAs) that show expression only in the tumor samples, with no or limited expression in the normal colon mucosa. These cancer signatures were found to be associated with dysregulation of biosynthetic machinery in the cancerous regions, and largely correlated with transcriptomics of the relevant glycosyltransferases revealing a CRC overexpressed biosynthetic pathway. Since TACAs are present in high abundance on the surface of cancer cells which are linked to many different proteins, these are very promising targets for the development of tumor-specific immunotherapy.