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Mini-review

Protein glycosylation in gastric and colorectal cancers: Toward cancer detection and targeted therapeutics

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ABSTRACT

Glycosylation is the most frequent and structurally complex posttranslational modification in cell-surface and secreted proteins. Glycans are major orchestrators of biological processes, namely, by controlling protein folding and key biological functions such as cell adhesion, migration, signaling and immune recognition. Altered glycosylation is considered a hallmark of malignant transformations that decisively contributes to disease outcome. This review comprehensively summarizes the main findings related with gastrointestinal cancers and the decisive impact of aberrant glycosylation on tumor biology toward more aggressive phenotypes. Particular emphasis is given to alterations in *O*-glycosylation, namely, the overexpression of immature *O*-glycans, and the sialylated Lewis antigens sialyl-LeA and sialyl-LeX, frequently implicated in lymphohematogenous metastasis. We further discuss how recent contributions from glycoproteomics and glycoengineering fields have broadened our understanding of the human *O*-glycoproteome and its implications for cancer research. Finally, we address the tremendous potential of glycans in the context of targeted therapeutics (selective inhibition of glycosylation pathways, immunotherapy) and discuss the need to include glycomics/glycoproteomics in holistic panomics models toward true precision medicine settings.

Introduction

This review addresses established and emerging glycan biomarkers for detection, prognosis and novel therapeutic strategies against gastric and colorectal carcinomas. These are among the most prevalent and deadliest gastrointestinal tract malignancies in western populations [1], mostly due to late diagnosis, rapid metastatic spread and scarce efficient therapeutics [2–4]. In fact, current treatments rely essentially on surgery associated with (neo)adjuvant chemotherapy, which is highly toxic and provides only modest results for advanced stage patients [2,5–7]. Over the past ten years the introduction of targeted therapeutics and immunotherapy has allowed significant, but yet insufficient, improvements in disease management [8–10]. As the oncology field moves toward precision medicine and patient-tailored therapeutic solutions, it becomes imperative to accomplish a more integrative and in-depth overview of the molecular nature of tumors [11–13].

Alterations in protein glycosylation are among the main molecular events accompanying oncogenic transformations in the gastric and colorectal tracts [14–16]. In fact, protein glycosylation is one of the most frequent, complex and plastic posttranslational modifications of membrane-bound and secreted proteins [17]. Glycans play a key role in protein folding, trafficking and stability. Moreover, they mediate several cell functions, such as cell adhesion, migration and signaling, as well as modulate immune recognition and host–pathogen interactions [16,18–20]. Protein glycosylation results from the highly coordinated action of nucleotide sugar transporters and sugar biosynthesis pathways, involving glycosyltransferases (GTs) and glycosidases in the endoplasmatic reticulum and the Golgi apparatus. As such, several factors may influence glycan biosynthesis, namely, the under or overexpression of glycosyltransferases, the impairment of glycosyltransferase chaperone function, altered glycosidase activity, changes in the tertiary conformation of a given peptide or growing glycan, and the availability of sugar nucleotide donors, cofactors and acceptor substrates

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[21,22]. The mislocalization of glycosyltranferases throughout the secreting organelles also contributes to significant alterations in cancer-associated protein glycosylation patterns [23–25]. Two main classes of glycans can be found at cell-surface glycoproteins: i) *O*-glycans, being the most common O-glycan that is initiated in the Golgi by the attachment of a GalNAc residue to the hydroxyl groups of serine (Ser) or threonine (Thr) amino acids of a given polypeptide chain (forming the Tn antigen GalNAcα-Ser/Thr, the simplest form of *O*-glycosylation) [26]; and ii) *N*-glycans, whose biosynthesis initiates in the endoplasmatic reticulum by the addition of an oligosaccharide chain to an asparagine (Asn) residue within consensus peptide sequences of Asn-X-Ser/Thr (X denotes any amino acid except proline) [27]. Less abundant forms of protein glycosylation include *O*-Fucosylation, *O*-GlucNAcylation, and *O*-Mannosylation [16]. Protein glycan chains are often branched or elongated and may present sialic acids, Lewis blood group related antigens or ABO(H) blood group determinants as terminal structures [28]. Other modifications may include phosphorylation, *O*-acetylation of sialic acids and *O*-sulfation of galactose and *N*-acetylglucosamine residues, thereby increasing the structural complexity of the glycophenotype [29]. In addition, protein glycosylation patterns do not follow a predefined template, as they are regulated by several factors at the cell and tissue levels, promptly responding to physiological and pathological changes [16].

Given its key functional and biological role, alterations in protein glycosylation underlying oncogenic transformations decisively contribute to the development of more malignant characteristics, such as cell–cell adhesion impairment, enhanced migration and promotion of lymphohematogenous metastasis [16,30–32]. Altered protein glycosylation has been also implicated in the activation of intracellular oncogenic pathways and immune escape, thereby favoring cancer-tolerogenic immune responses [33,34]. Particularly, advanced stage tumors often overexpress or promote the *de novo* biosynthesis of immature and truncated *O*-glycans, such as the Tn, sialyl-Tn (STn), T and sialyl-T (ST) antigens, due to a premature stop of the extension of *O*-glycosylation [35–37]. Oversialylation and fucosylation of glycan chains are also frequently observed in cancer, including terminal antigens like the sialyl-LeA (SLeA) and sialyl-LeX (SLeX) [38–40]. Contrasting with the tumor, these structures are often absent or just moderately expressed in the corresponding healthy tissues, holding potential for selective targeted therapeutics [38]. In addition, many of the proteins carrying cancer-associated glycans may also be shed into the bloodstream or other bodily fluids, facilitating non-invasive detection methods.

Despite the key role played by glycosylation, clinically approved and novel targeted therapeutic approaches for gastric and colorectal tumors have mostly resulted from intense genomic, transcriptomic and proteomic studies. Moreover, few efforts have been devoted to whole glycome and glycoproteome characterization of gastric and colorectal tumors, mostly due to its intrinsic molecular complexity. This has significantly delayed the development and translation of glycan-based diagnostic and therapeutic solutions to clinical routine. Recently, the simplification and standardization of glycobiology-based methods have provided powerful analytical tools to improve our understanding of glycosylation alterations on specific cancer-associated proteins. This review summarizes recent insights from innovative research on the glycobiology of gastric and colorectal tumors. It emphasizes the *O*-glycome and glycoproteome, envisaging the identification of more specific cancer glycobiomakers and the development of innovative therapeutic strategies. Furthermore, it comprehensively discusses the implications of combining glycosylation, large scale genomics, transcriptomics and metabolomics toward true precision medicine settings.

Protein glycosylation in gastrointestinal cancer: molecular mechanisms underlying the aberrant glycan biosynthesis

Perhaps the most studied cancer-associated glycoepitopes in gastric and colorectal cancers derive from a premature stop in the elongation of protein *O*-GalNAc glycosylation [16,37,41]. These antigens have been classically termed as simple mucin-type *O*-glycans, reflecting the abundance of this type of glycosylation in mucins. Nevertheless, these types of glycans may be virtually found in any membrane bound and secreted protein expressing *O*-glycosylation sites. *O*-GalNAc glycan biosynthesis can be initiated by up to 20 polypeptide GalNAc transferases (GalNAc-Ts), which are responsible for catalyzing the transfer of a *N*-acetylgalactosamine residue from UDP-GalNAc to the hydroxyl group of Ser or Thr, originating the Tn antigen (Fig. 1) [42–44]. The different GalNAcTs present a cell and tissue-specific expression [45], showing distinct and partially overlapping peptide substrate specificities that are crucial for *O*-glycosites definition [46]. There have been reports of an increased density of *O*-glycans in gastric and colon tumors, resulting from an increased GalNAc-Ts activity in tumor cells compared to normal cells [20,47]. In most normal gastrointestinal cells, the Tn antigen is further elongated by core 1 β1,3-galactosyltransferase (C1GalT). This reaction originates the core 1 or Thomsen–Friedenreich (T)-antigen (Galβ1-3GalNAc-Ser/Thr), in a process dependent on the functional chaperone COSMC (Fig. 1) [48]. The initial GalNAc may be extended and originate the core 3, catalyzed by β-1,3-*N*-acetylglucosaminyltransferase 6 (β3Gn-T6). Core 3 may be further substituted with a GlcNAc residue by core 3 β-1,3-N-acetylglucosaminyltransferase (C3GnT), originating core 4 (Fig. 1). On the other hand, core 1 may originate core 2, catalyzed by β-1,6-*N*-acetylglucosaminyltransferases (C2GnTs). Core structures are frequently further elongated and terminated with ABO and Lewis blood groups determinants, as depicted in Fig. 1. The downregulation of β3Gn-T6 and C3GnT was shown to suppress metastasis in colon carcinoma, suggesting that core structures may play a key role in cancer progression [49]. C1GalT is often overexpressed in tumors, resulting in an accumulation of T antigens, which has been associated with disease progression, metastasis and decreased survival [20,38]. Early sialylation also decisively contributes to a premature stop in *O*-glycan extension, leading to an accumulation of immature sialylated structures such as sialyl-Tn (STn; Neu5Acα2-6GalNAcα-O-Ser/Thr), mostly due to the increased expression of sialyltransferases like ST6GalNAc1 [37,50]. The overexpression of Tn and STn has been observed in both early and advanced stage disease, generally associated with poor outcome [51,52]. Notably, pre-malignant and early stage colorectal tumors overexpress the STn antigen [53–55] due to a reduction in *O*-acetylation of sialic acids [53,56,57] responsible for protecting colonic mucins from degradation by intestinal bacteria [58]. Nevertheless, the molecular mechanisms underlying these transformations are not yet fully understood. Other modifications occurring in sialic acids may include a substitution of Neu5Ac by non-human Neu5G from dietary sources [59]. Recently, high levels of Neu5Ac were associated with increased consumption of red meat and as a promoter of systemic inflammation and cancer [60]. Furthermore, several studies report an overexpression of the T antigen sialylated form in colorectal carcinomas [61], whose contribution to disease warrants in depth investigation.

In addition, gastric and colorectal tumors present high levels of SLeA (NeuAcα2,3Galβ1,3[Fucα1,4]GlcNAc-R) and SLeX (NeuAcα2,3Galβ1,4[Fucα1,3]GlcNAc-R) as terminal epitopes of protein *O*-glycans [62,63], but also of *N*-glycans and glycolipids [64,65]. In fact, SLeA and SLeX have been found to be highly expressed in many solid tumors, including digestive track carcinomas, and their expression levels have been correlated with metastasis and poor survival in cancer patients [66–68]. These antigens are structurally