Cadherins Glycans in Cancer: Sweet Players in a Bitter Process

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ABSTRACT

Cadherins are key components in tissue morphogenesis and architecture, contributing to the establishment of cohesive cell adhesion. Reduced cellular adhesiveness as a result of cadherin dysfunction is a defining feature of cancer. During tumor development and progression, major changes in the glycan repertoire of cancer cells take place, affecting the stability, trafficking, and cell-adhesion properties of cadherins. Importantly, the different glycoforms of cadherins are promising biomarkers, with potential clinical application to improve the management of patients, and constitute targets for the development of new therapies. This review discusses the most recent insights on the impact of glycan structure on the regulation of cadherin function in cancer, and provides a perspective on how cadherin glycans constitute tumor biomarkers and potential therapeutic targets.

Cadherin Glycans: More Than Sweet Molecules in Cancer

Loss of cell adhesion is a major molecular event occurring during malignant transformation [1]. Concomitantly, cancer cells suffer profound alterations in the biological repertoire of glycans attached to the cell surface and/or expressed in the cytoplasm [2]. Glycans exert a powerful effect on cadherins by influencing their biophysical properties, homotypic interactions, and cell adhesion functions. The type of glycan structure has a differential impact on cadherin-mediated cell adhesion, thereby regulating cancer cell fate. For example, cadherins bearing branched complex N-glycans are less competent in terms of cell adhesion and are associated with cancer progression. By contrast, the expression of bisecting glycans on cadherins strengthens cell adhesion and is associated with cancer suppression. In this review we present the most recent evidence of the roles that different glycans play in the regulation of cadherin function in cancer, and discuss applications in oncology.

Cadherins

The cadherin superfamily (see Glossary) comprises a set of calcium-dependent adhesion molecules that participate in cell–cell interactions and determine cell polarity and tissue archi- tecture during embryonic morphogenesis and homeostasis [3,4]. These adhesion glycoproteins organize into lateral clusters at the cell surface where they mediate homophilic contacts between neighboring cells and participate in dynamic interactions with the actin cytoskeleton as core components of the adherens junctions (Box 1) [5]. In fact, classical cadherins are membrane- spanning macromolecular complexes that serve as important mechanosensors at cell–cell junctions, modulating cell behavior during tissue remodeling and cellular transformation [6,7].

Dysfunction of cadherins is an important determinant of cancer development and progression [1,8]. The disruption of cadherin-mediated cell adhesion leads to disorganized tissue architecture [9,10]. Because many solid tumors are of epithelial origin, epithelial cadherin (E-cadherin or cadherin 1) is an attractive target in cancer. E-cadherin is a tumor suppressor [11] that can be dysregulated by multiple mechanisms including loss of heterozygosity [12,13], inherited and somatic mutations [14,15] (well characterized in gastric cancer), promoter hyper- methylation [16,17], transcriptional silencing [18], endocytosis [19,20], and proteolysis [21]. However, a high percentage of human epithelial invasive cancers with dysfunction of E-cadherin- mediated cell adhesion do not have

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genetic and structural alterations in the protein, and the underlying mechanism of cadherin dysregulation remains unknown [22]. This gap in knowledge has fuelled the identification of other molecular mechanisms of (dys)regulation of cadherins in cancer. Glycosylation has been pinpointed as a fundamental mechanism controlling the adhesion functions of cadherins, and has potential to be targeted in cancer therapy [23,24].

Protein glycosylation is an important post-translational modification in eukaryotes, where more than half of all proteins are glycosylated [25]. The structural diversity of glycans that can be added to proteins – known as the glycome or the glycan repertoire – contributes to a wide range of biological functions [26,27]. In cancer, cellular transformation is typically accompanied by changes in the glycome, and aberrant glycosylation is considered to be a hallmark of neoplastic cells [2,28–30].

Decoding the function of cadherin glycans in cancer has been revealed to be fundamental for further understanding cancer cell biology and to potentially improve the clinical management and treatment of patients.

Box 1. E-Cadherin Structure

The immature form of E-cadherin is a polypeptide composed of a propeptide sequence of about 130 amino acids (aa) and a mature polypeptide of about 728 aa. The propeptide corresponds to a short signal sequence for import into the ER where it undergoes cytoplasmic trimming [11]. Following this trimming process, mature E-cadherin is routed towards the basolateral surface of epithelial cells. E-cadherin mature protein is organized into three major structural domains: an N- terminal ectodomain of about 550 aa, a single transmembrane domain, and a short C-terminal cytoplasmic domain of about 150 aa [11]. The ectodomain comprises five extracellular cadherin (EC) repeat domains that mediate adhesive (trans) interactions with cadherins embedded in opposing membranes, which then undergo lateral (cis) interactions with adjacent cadherin molecules [136,137]. The cadherin cytoplasmic domain interacts with the so-called 'core cadherin– catenin complex' (b-catenin, p120-catenin, and many others) which in turn provides anchorage to the actin cytoskeleton to form stable cell–cell contacts. The dynamic cooperation of cadherin–catenin complexes with the cytoskeleton contributes to both the stability and plasticity of adherens junctions [138–140].

Glycosylation: Key Mechanism in Cadherin-Mediated Cell Adhesion.

The regulatory power of glycans is evident in the early secretory pathway of cadherins. Glycosylation of cadherins starts in the endoplasmic reticulum (ER) while the proteins emerge from the translocon complex (Box 2) [31]. The addition of a core-oligosaccharide structure to asparagine (Asn) confers thermodynamic stability [32], guides correct folding [33], and ensures structural assembly and stability of the proteins [34]. The trafficking of cadherins to the Golgi is also controlled by glycosylation through the cytoplasmic modification of cadherin with O-linked N-acetylglucosamine (O-GlcNAc) structures (Figure 1) [35]. The O-GlcNAcylation modification of E-cadherin is associated with its retention in the ER by interfering with E-cadherin binding to p120-

catenin and type I g-phosphatidylinositol phosphate kinase (PIPKIg), a protein required for recruitment of E-cadherin to adhesion sites [36].

Glycans also dictate spatial cadherin arrangement at the cell membrane and contribute to homotypic cellular interactions. E-cadherin modified with high-mannose N-glycans is predominantly expressed at the cell surface and more effectively enhances cell–cell interactions than does E-cadherin modified with hybrid and complex N-glycans [37–39]. Interestingly, glycans modulate lateral (cis) interactions between neural cadherins (N-cadherins or cadherin 2) on the same cell, thereby governing the dynamics of intercellular junction assembly [40–42]. It is important to mention that this dynamic contribution of glycans to the regulation of cadherin- mediated cell adhesion is known to be cell- and tissue-specific.

Recent reports have identified cadherins as major carriers of O-linked mannose structures, and these O-mannose glycans on cadherins are not further elongated (Figure 1) [43,44]. O-mannosylation-deficient embryos exhibit defects in the molecular architecture of cell–cell contact sites and are arrested at the morula-to-blastocyst transition stage [45]. Conserved O-mannosylation sites in T- (cadherin 13, H-cadherin), E-, and N-cadherins across different species suggest a general role of O-mannosyl glycans in the regulation of cadherin-mediated cell adhesion [43,46]. In fact, inhibition of O-mannosylation leads to compromised cadherin- mediated adhesion [45].

Regarding N-glycosylation, there are four potential N-glycosylation sites (Asn-554, Asn-566, Asn-618, and Asn-633) in the ectodomain of E-cadherin (Figure 1), and the occupancy of each site by a specific N-glycan depends on the cell, tissue, and pathophysiological context [47]. Proliferating epithelial cells (sparse cell cultures) display E-cadherin modified with complex type N-glycans, leading to unstable adherens junctions, while differentiated cells with mature adhe- rens belts (dense cell cultures) have E-cadherin modified with hybrid/high-mannose oligosaccharides [48]. N-glycosylation also influences the molecular organization of adherens junctions and anchorage to the actin cytoskeleton [48,49], indirectly impacting on the assembly of tight junctions [50]

Box 2. Protein Glycosylation in Homeostasis and Cancer

Glycomics defines the structure and functional roles of glycans in complex biological systems and can be applied to understanding how post-translational modifications via glycosylation affect cancer development and progression. The mammalian glycome repertoire is estimated to be 10–104 times larger than the proteome, and is far more complex than either the genome or the proteome [141]. Glycosylation consists of the covalent attachment of carbohydrate structures to proteins and lipids in the endoplasmic reticulum (ER) and Golgi, producing different families of glycoconjugates [2]. In proteins, glycans are usually attached to the polypeptide backbone via N- or O- linkages.

The N-linked glycans are attached to asparagine (Asn) residues of nascent proteins within the consensus peptide sequence Asn-X-Ser/Thr, where X is any amino acid except proline [82]. N-linked glycans are further extended and elongated in the Golgi compartment by several glycosyltransferases that operate in a stepwise manner. N-glycosylation initiates at the ER membrane where $Glc_3Man_9GlcNAc_2$ is synthesized and covalently coupled to the polypeptide backbone. This en bloc transfer is catalyzed by oligosaccharyltransferase (OST), a multisubunit protein complex associated with the translocon complex. At the ER, glycans have a common role in protein folding, quality control, and some sorting events [33]. Once glycoproteins are folded and oligomerized correctly, they move to the Golgi, where N-glycans are further extensively modified [82,142]. During elongation and extension at the trans-Golgi, several carbohydrate modifications can occur: (i) core fucosylation, mediated by Fut8; (ii) elongation of branch N-acetylgluco- samine (GlcNAc) residues of N-glycans (e.g., poly-N-acetyllactosamine, polyLacNAc); or (iii) or 'capping' and 'decoration' of elongated branches (the addition of sialic acid, Neu5Ac; fucose, Fuc; galactose, Gal; or N-acetylgalactosamine, GalNAc) [82].

Cancer cells exhibit a predominance in b1,6-GlcNAc residues (catalyzed by N-acetylglucosaminyltransferase V, GnT-V) that are frequently extended with polylactosamine structures (Galb1,4GlcNAcb1,3, a ligand for galectins), which in turn are terminally modified with sialic acid (Figure I). These glycan structures impair the function of cadherins and promote malignant and invasive phenotypes. Normal/benign cells tend to display E-cadherin modified with bisecting GlcNAc residues (catalyzed by N-acetylglucosaminyltransferase III, GnT-III), with negligible levels of branched glycans.

O-linked glycosylation consists in the attachment of carbohydrate moieties to serine (Ser) or threonine (Thr) residues [82]. Mucin-type O-glycosylation consists of the attachment of GalNAc structures to Ser or Thr residues [143]. O-mannosyla- tion is initiated by the covalent attachment of mannose to Ser or Thr residues of secretory and membrane proteins in the ER lumen, catalyzed by the protein O-mannosyltransferases 1 and 2 (POMT1 and POMT2) [144]. The O-mannose core structures can be further extended via GlcNAcb1–2Man, GlcNAcb1–4Man, or GlcNAcb1–6Man linkages [145]. In homeostasis, cadherins are O-mannosylated. During malignant transformation cadherins show decreased O-manno- sylation concomitant with increased branched structures [87].

Several factors may affect glycan heterogeneity in proteins: the expression and localization of glycosyltransferases in the ER/Golgi complex, the ratio of enzymatic activities, enzyme accessibility to substrate, nucleotide sugar metabolism, and Golgi pH [2,29], and the variability of glycosylation patterns precludes the precise prediction of glycans in a particular cell type.

Owing to the complexity and dynamic nature of glycans, their biological roles are varied and range from the folding of nascent proteins and intracellular trafficking to roles in molecular and cellular homeostasis [146], cell adhesion and cell signaling [147], immune modulation, and endocytosis [148,149].

Glycosylated Cadherins in Cancer Development

The glycosylation pattern of cadherins undergoes profound modifications during the acquisition of the malignant phenotype (Figure 2, Key Figure) [28,51]. The most-common glycosylation changes are altered core fucosylation, highly branched N-glycans, increased terminal sia- lylation, and altered expression of O-mannose structures [23,29].

Core fucosylation consists of the addition of /1,6-fucose to the innermost N-acetylglucosamine (GlcNAc) residue of the oligosaccharide core, catalyzed by /-1,6- fucosyltransferase (Fut8) (Box 2). In gastric cancer, core fucosylation is downregulated, and its upregulation was found to inhibit cell proliferation of human gastric cancer cells [52,53]. In colon cancer cells, introduction of Fut8 results in increased expression of E-cadherin at the cell surface [54]. Evidence suggests that addition of /1,6-fucose to E-cadherin reduces tyrosine (Tyr)-654 phosphorylation of b-catenin, leading to reduced accumulation of nuclear b-catenin and thus to increased binding affinity to E-cadherin and enhancement of cell–cell adhesion [55]. Recently, it was reported that decreased core fucosylation of E-cadherin in lung cancer cells activates Src kinase, inducing an upregulation of N-cadherin and Snail1, two molecular drivers of the epithelial–mesenchymal transition (EMT) [56]. However, the role of core fucosylation in the regulation of E-cadherin function remains controversial because increased core fucosylation of E-cadherin is also observed during lung cancer progression [57].

b1,6-N-Acetylglucosaminyltransferase-V (GnT-V) is involved in the biosynthesis of the so-called 'b1,6-GlcNAc branch' structure of N-glycans (Box 2) [58], and is highly implicated in tumor progression [51]. In fact, transcription of MGAT5 (gene encoding GnT-V) is upregulated by oncogene signaling [58,59]. Several types of cancer exhibit overexpression of b1,6-GlcNAc branched N-glycans, such as gastric [60], colon [61], and breast tumors [62]. GnT-V expression impairs epithelial cell contact inhibition [63], increases cell proliferation in normal mammary epithelial cells [64], and modulates the canonical Wnt/b-catenin and Her-2-mediated signaling pathways [65]. Suppression of GnT-V activity results in reduced levels of tumor-initiating cells and tumorigenic potential in vivo and in vitro [66,67], as wells as delayed tumor onset [64]. Further extension of b1,6-GlcNAc branched N-glycans with poly-N-acetyllactosamine structures potentiates their binding to galectins (a family of carbohydrate-binding proteins) that generate the lattices (galectin–glycan structures) controlling the surface retention time of gly- coproteins [68,69]. Modification of E-cadherin with b1,6-GlcNAc branched N-glycans affects its cell-membrane expression [70] and the molecular assembly and stability of adherens junctions [71] by interfering with the recruitment of b-catenin and p120-catenin, thereby compromising their clustering capacity at the cell surface [72] and reducing the cellular adhesion [73] (Figure 2). Branched N-glycosylation on N-cadherin affects the outside-in signal transduction pathway of ERK and reduces homotypic cell–cell adhesion [42,74].

Glycosylation of cadherins during cancer development occurs in a site- and context-specific manner. Each site can be differently glycosylated, not all glycosylation sites are occupied, and not all sites are important for cadherin-mediated adhesion in all contexts. For instance, in human breast carcinomas, all four potential N-glycosylation sites of E-cadherin are glycosylated and each N-glycan has distinctive functions. N-glycans at Asn-554 and Asn-566 affect cell-cycle progres- sion and proliferation through the activation of the ERK signaling pathways [75], as well as adhesion and the composition of adherens junctions [76]. Removal of specific N-glycans from E- cadherin increases tyrosine phosphorylation of b-catenin, destabilizing the adhesion complex [76]. In oral cancer, hyperglycosylation at Asn-554 with complex N-glycans, as a result of overexpression of DPAGT1 and activation of Wnt signaling, reduces cell adhesion and com- promises the intercellular junctions [77,78]. Glycan at Asn-633 is important for E-cadherin expression, folding, and trafficking to the Golgi because deglycosylation at this site specifically arrests E-cadherin in the calnexin/calreticulin quality-control cycle at the ER, where it is recognized as a misfolded protein and then degraded via the ER-associated protein degradation (ERAD) pathway [79]. In gastric cancer cells, only Asn-554 and Asn-633 were demonstrated to be modified with b1,6-GlcNAc branched N-glycans and high mannose/hybrid N-glycans, respec- tively. Interestingly, preventing glycan modification at Asn-554, by blocking GnT-V-mediated N- glycosylation, resulted in increased expression of E-cadherin at the cell surface and increased cis- dimerization capacity, thereby having a protective effect on E-cadherin adhesion function [72].

Overexpression of sialylated structures is another cancer glycophenotype [80]. The addition of sialic acid residues, which frequently terminate the carbohydrate chains, is mediated by sialyltransferases, a family of glycosyltransferases frequently upregulated in cancer [81]. Sialic acids can be attached to subterminal sugars such as N-acetylgalactosamine or N-acetylglucos- amine (through an /2–6 bond), to galactose (through /2–3 or /2–6 bonds), or to another terminal sialic acid (through an /2–8 bond), thereby generating polysialic acids [82,83]. The position of sialylated antigens on the cell membrane can regulate glycoprotein conformation, clustering, and cell–cell interactions such as adhesion. Electrostatic repulsion of negative charges on sialic acids disrupts cellular adhesion by breast carcinoma cells [84,85] and promotes their tumor-initiating potential [86].

Cancer cells are also characterized by reduced protein O-mannosylation. Loss of O-mannosyl glycans attached to E-cadherin affects E-cadherin expression and compromises cadherin– catenin interactions. Interestingly, reduced GnT-V-mediated N-glycosylation of E-cadherin is accompanied by increased O-mannosylation [87].

Glycosylated Cadherins in Invasion and Metastasis

Functional changes in the glycan repertoire play significant roles in oncogenic transformation by potentiating invasion and the metastatic behavior of cancer cells [2]. Despite the huge diversity of glycans, only a specific set of cadherins glycoforms are associated with metastasis (Figure 2).

O-GlcNAcylation modification is linked to cellular features relevant to metastasis in ovarian and prostate cancer cells [88,89]. In fact, increased O-GlcNAcylation in cancer reduces the expression of E-cadherin, suggesting increased retention of E-cadherin in the ER. Core fucosylation and sialylation are significantly increased in the serum N-glycome of cancer patients [90], and the

increase is associated with invasion [91,92]. The fucosylation index of E-cadherin is considered as a potential prognostic marker of metastatic lung adenocarcinoma [57]. Expression of sialyl Lewis X (SLe x) in cancer is associated with invasion and poor outcome, and is accompanied by reduced levels of E-cadherin [84,93–95] and an altered pattern of E-cadherin localization in pancreatic adenocarcinoma cells [96].

GnT-V is upregulated in invasive/metastatic cancer cells. GnT-V substrate proteins are a unique subset of coexpressed tumor markers associated with poor outcome in cancer patients [97]. In fact, downregulation of GnT-V in gastric cancer cells was described to inhibit metastasis via an EGFR signaling-initiated EMT phenotype and MMP-9 expression [60,98]. Expression of other metalloproteinases is also modulated by GnT-V, and is thus associated with increased invasive potential of colon cancer cells [99,100]. By contrast, upregulation of GnT-V increases the expression of the EMT-associated factors snail, twist, and N-cadherin, and induces changes in E-cadherin localization [101]. Specific modification of E-cadherin with b1,6-GlcNAc branched N-glycans in invasive gastric carcinomas correlates with poor survival rates of patients [72].

Glycosylated Cadherins and Cancer Suppression

N-Acetylglucosaminyltransferase-III (GnT-III), encoded by MGAT3, catalyzes the addition of a GlcNAc residue to the b-mannose via a b1,4 linkage [58,102] (Box 2). The resulted 'bisecting GlcNAc' structure impacts on the activities of other glycosyltransferases by restricting the N- glycan conformation [103,104], thus preventing further processing and elongation of N-glycans such as GnT-V-mediated branching [105,106]. GnT-III activity was reported to antagonize GnT-V effects on cell survival and metastasis. Whereas GnT-III (Mgat3)-deficient mice showed increased tumor growth and metastasis [107], upregulation of GnT-III in highly-metastatic mouse melanoma cells precludes GnT-V activity and results in significant suppression of cancer metastasis [108]. This suppressive role was attributed to GnT-III-mediated N-glycosylation of E-cadherin. The overexpression of GnT-III leads to increased modification of E-cadherin with bisecting GlcNAc N-glycans, and this stabilizes the localization of E-cadherin at the cell surface [70] and delays its turnover rate and endocytosis [71]. As a result, the phosphorylation levels of b-catenin decrease and binding to E-cadherin at the cell surface is promoted [109], further contributing to stabilization of the adhesion complex and enhancement of intercellular binding [110].

On the other hand, E-cadherin-mediated adhesion complexes regulate the cellular levels of GnT- III. GnT-III expression is upregulated by increased cell–cell interactions via stable E-cadherin– catenin– actin complexes [70,111,112], and is downregulated when b-catenin translocates to the nucleus and activates Wnt signaling [113]. TGF-b-mediated EMT is also implicated in the regulation of GnT-III by promoting hypermethylation of MGAT3 and decreased expression and activity of GnT-III in epithelial cell lines [114,115].

Cadherin Glycans: Promising Tumor Biomarkers

Tumor biomarkers are urgently needed to improve early diagnosis, prognosis, risk stratification, to predict therapeutic response, and to fuel the development of new drug targets and therapies [116]. Early dysregulation of E-cadherin-mediated cell–cell adhesion is a prominent alteration at the

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invasive fronts of many epithelial cancers, and is considered to be an early molecular event in diffuse-type gastric carcinoma [117] and lobular breast cancer [118]. Therefore, the underlying molecular alterations of cadherins dysfunction are promising biomarkers of tissue transformation and cancer progression. Given the profound alterations of the cadherin glycosignature and their correlation with the clinicopathological features of cancer patients, the clinical assessment of cadherin glycoforms either in tumor biopsies or in serum provides highly-specific glyco-based biomarkers for improving the clinical management of patients (Figure 2). As an example, it is currently possible to detect E-cadherin specifically modified with b1,6-GlcNAc branched N- glycans by in situ proximity ligation assay in gastric cancer biopsies [71,72]. Further correlation with the clinicopathological features revealed an association between this cadherin glycoform and poor prognosis of cancer patients [72].

Moreover, soluble cadherins resulting from the shedding of the cadherin ectodomain can also be detected in serum [119]. As a result of alterations in cellular topography during cancer cell transformation [120], soluble E-cadherin is constitutively shed at higher levels at tumor sites and metastatic foci [121]. Chan et al. showed that soluble E-cadherin levels were significantly elevated in patients with gastric cancer compared to healthy individuals [122]. Therefore, soluble E-cadherin specifically modified with b1,6-GlcNAc branched N-glycans can be used as a specific and non-invasive biomarker for the early detection of cancer and for monitoring tumor recurrence and therapeutic responses.

Glycomics and glycoproteomics-based technologies have been growing fast and are available to improve personalized medicine [123,124]. Rapid screening methods have been used to analyze glycosylation patterns on glycoproteins in large cohorts of patients, enabling the identification of a new generation of disease (glyco)biomarkers [125]. Profiling glycans in serum can be carried out by advanced (glyco)technological approaches such as mass spectrometry [126], lectin array [127–129], or via specific anti-glycan antibodies [130]. In addition, tumor-associated carbohy- drate antigens are known to elicit an immune response, raising the levels of serum antibodies that can be detected by printed glycan arrays [131]. Furthermore, glycan microarrays have important biomedical applications, including the characterization of potential inhibitors of glycosyltransfer- ases and glycosidases and new antagonists targeting specific lectin receptors [132,133]. Inte- grating these high-throughput glycotechnology methods in the clinical identification of cadherins glycoforms will certainly contribute to the discovery of a new era of cancer biomarkers paving the way for the development of new glycoprotein-based therapeutic drugs.

Concluding Remarks and Future Directions

Tumor development and progression are often associated with cadherin dysfunction and transition to a more motile and invasive phenotype. Glycosylation is a major post-translational modification of proteins that controls the biological function of cadherins in cancer. Under- standing how different glycan structures regulate and control the folding, trafficking, and cell- adhesion functions of cadherins will contribute greatly to elucidating the molecular pathogen- esis of cancer. With the advent of new and cutting-edge glycotechnological approaches able to detect different protein glycoforms with high specificity and sensibility [134], the specific detection of cadherin glycoforms in the serum of cancer patients constitutes a valuable non-invasive biomarker of prognostic value to be included in the clinical algorithm for cancer patients. Particularly, in diffuse-type gastric cancer

and lobular breast cancer, where early alterations of E-cadherin triggers cancer development, the early in situ detection of cadherin alterations by assessing cadherin glycoforms is of great interest in improving early diagnosis and risk stratification of patients. Taken together, the detection of cadherin glycoforms is of major interest in oncology not only as a prognostic biomarker but also as potential target for the development of new therapies (see Outstanding Questions). Blocking the addition of the deleterious glycans, such as b1,6-GlcNAc branched N-glycans or sialylated glycans, on cadherin by using molecules targeting specific glycosyltransferases expressed in specific cells [135] constitutes an important strategy for modulating cancer cell behavior. Moreover, in the era of cancer immunotherapy, anticancer vaccines targeting tumor-associated carbohydrate antigens (such as cadherin glycoforms) provide another appealing option for boosting antitu- mor immunity. Activation of an antitumor immune response against cadherins bearing branched/sialylated glycans is foreseen as potential strategy for triggering tumor cell elimination.

Acknowledgements

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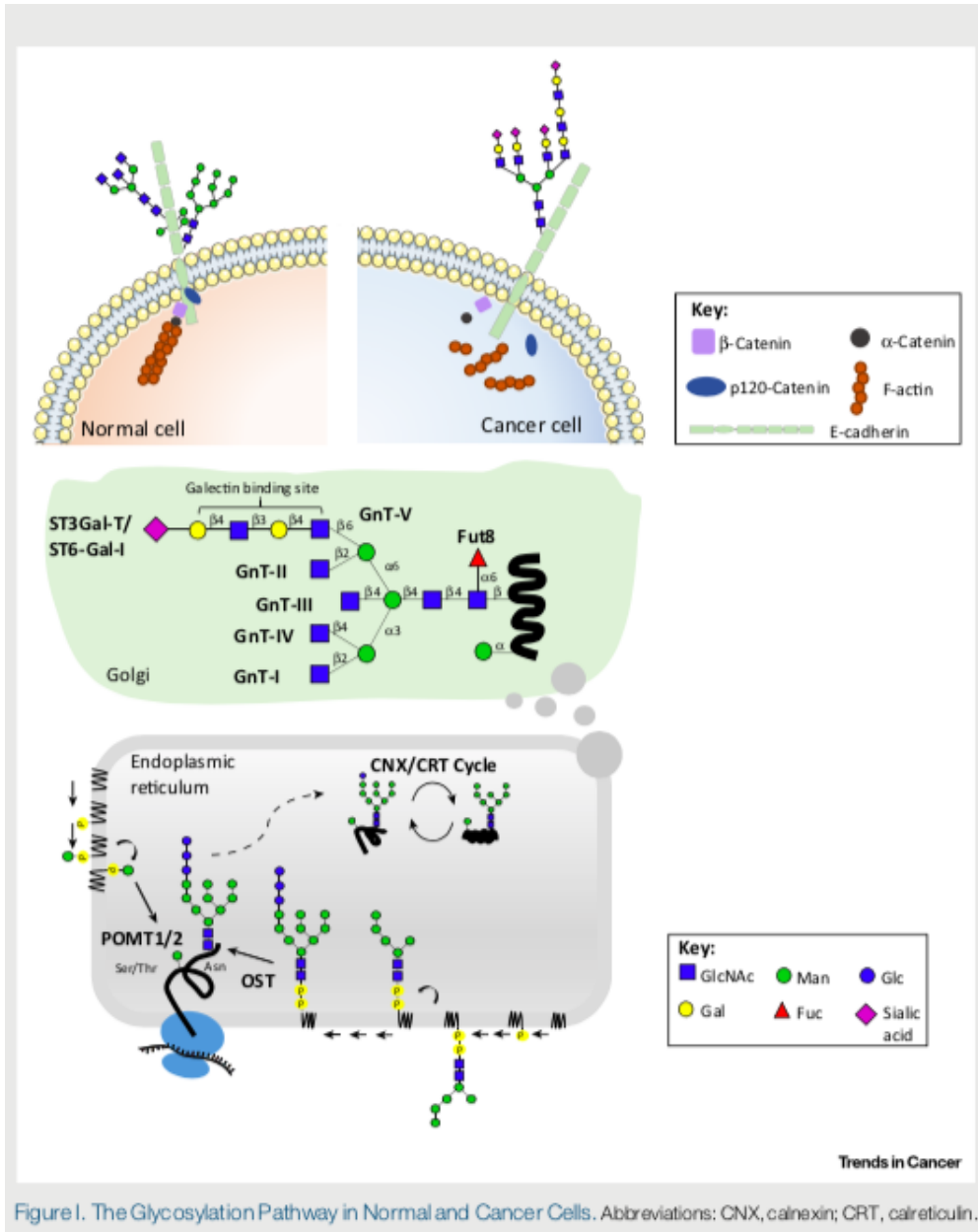
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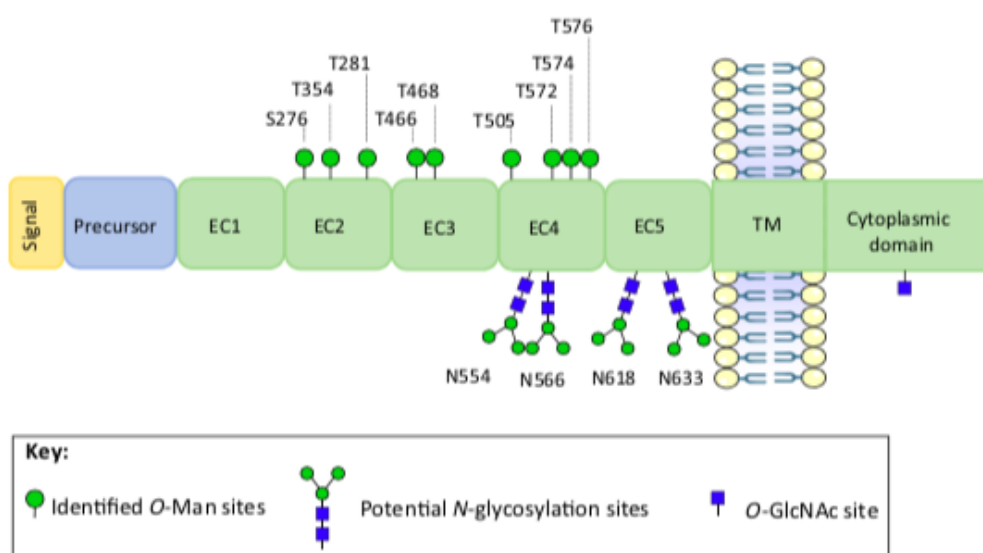
Figure I. The Glycosylation Pathway in Normal and Cancer Cells. Abbreviations: CNX, calnexin; CRT, calreticulin.

Figure 1. Schematic Representation of Classical Cadherin Structure and Glycosylation Sites. The major forms of cadherin glycosylation that impact upon its cell-adhesion functions in cancer are N-glycans, O-mannosyl glycans, and O- GlcNAc structures. The human E-cadherin ectodomain comprises four potential N-glycosylation sites: two putative sites located in the EC4 subdomain (Asn-554 and Asn-566), and two sites in the EC5 subdomain (Asn-618 and Asn-633). The N-glycosylation of E-cadherin contributes to up to 20% of its total mass, and glycosylation site occupancy is dependent on cell, tissue, and pathophysiological context. The O-mannose glycoproteome recently identified classical cadherins as major carriers of O-linked mannose structures attached to potential and predicted glycosites localized in the EC2–5 domains. Moreover, O-GlcNAc glycans were also described to modify E-cadherin in its cytoplasmic domain, thereby interfering with its folding, trafficking, and consequently cell-adhesion functions. Abbreviations: EC, extracellular domain; O-GlcNAc; O- linked N-acetylglucosamine; O-Man, O-linked mannosylation; TM, transmembrane domain.

Key Figure

Changes in Cadherin Glycosylation during Cancer Development and Progression: A Promising Biomarker
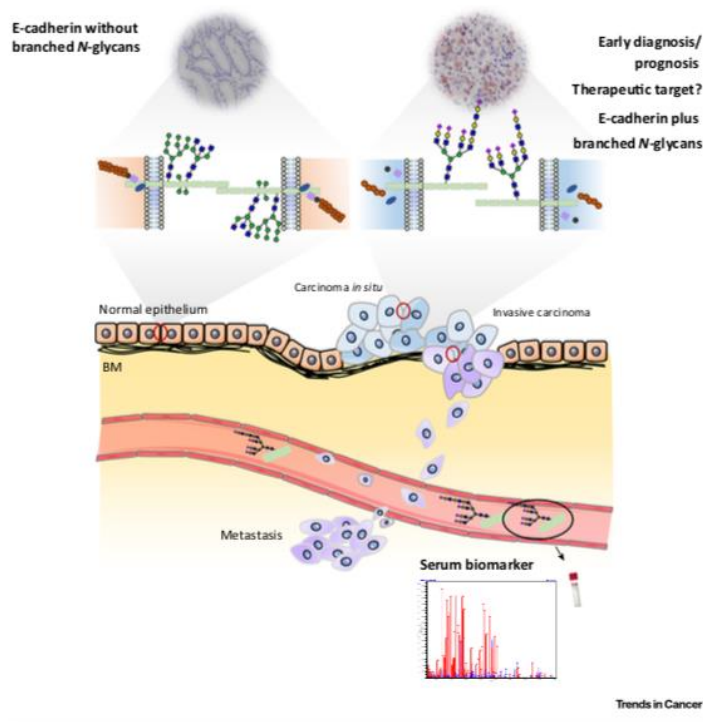
Figure 2. For a Figure360 author presentation of Figure 2, see the figure online at http://dx.doi.org/10.1016/j.trecan.2016. 08.003#mmc1. Modifications in the glycosylation of cadherins occur during cellular transformation, invasion, and metastases. In home- ostasis and in normal/suppressive contexts, where cell–cell adhesion is preserved and adherens junctions are competent, E-cadherin is predominantly modified with bisecting N-glycans, high-mannose N-glycans, and O-mannosyl glycans. During neoplastic transformation, E-cadherin is highly modified with b1,6-GlcNAc branched N-glycans, further extended with poly- LAcNAc structures and terminal sialylation, that promote malignant and invasive phenotypes. These different glycoforms of cadherins are hallmarks of either 'normal' or cancer phenotypes, and can be used as biomarkers. The detection of cadherins specifically modified with b1,6-GlcNAc branched N-glycans in a biopsy sample or, as an non-invasive approach, in serum (by proximity ligation assay technology or glycan array) supports its applicability in cancer early diagnosis, determination of prognosis, and patient risk stratification. Abbreviation: BM, basement membrane.