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Review

Glycosylation and Integrin Regulation in Cancer

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Integrins are transmembrane receptors that coordinate extracellular matrix (ECM)–cell and cell–cell interactions, signal transmission, gene expression, and cell function. The aberration of integrin function is one of the well-recognized mechanisms of cancer. The activity of integrins is strongly influenced by glycans through glycosylation events and the establishment of glycan-mediated interactions. Glycans represent a class of ubiquitous biomolecules that display an extraordinary complexity and diversity in both structure and function. Widely expressed both in the ECM and on the cell surface, they play a crucial role in mediating cell proliferation, survival, and metastasis during cancer. The purpose of this review is to provide an overview of how both glycosylation of integrins and integrin interaction with the cancer glyco-microenvironment can regulate cancer progression.

Integrins and Glycans: Important Players in Cancer

Integrins (see Glossary) are transmembrane receptors that bind extracellular matrix (ECM) components and trigger signalling cascades that regulate cellular events during development, normal homeostasis, and disease [1]. In 1995, Meyer and Fässler showed that the knockout of β1 prevented the implantation of mouse embryos *in utero* since the majority of ECM-binding integrins were suppressed, proving their essential role in cellular physiology [91]. Integrins consist of two non-covalently linked subunits: α and β (Figure 1A). In mammals, 18α- and 8β-integrin isoforms combine into 24 αβ receptors that act as a bridge between the cell and the ECM in transducing bidirectional information (Figure 1B) [2]. Integrins bind a wide variety of ligands: they recognize and bind several ECM components such as collagens, laminins, and fibronectins; and they also mediate cell–cell interactions by binding other cell receptors or soluble molecules (Figure 1C) [3]. Integrins have a well-recognized role in cancer, controlling cell migration, metastasis, and chemoresistance mechanisms (Box 1) [4,5]. Integrin function is regulated by a wide variety of molecular events that includes glycosylation. Cells are covered with a dense and large array of sugars and the ECM of eukaryotes is rich in glycan-based structures (Figure 2) [6]. The glyco-microenvironment involves several glycan-based post-translational modifications ranging from branched structures (N and O linked) to linear polysaccharides [**glycosaminoglycans (GAGs)**] of cell surface and ECM proteins (Box 2). These glyco-components are mutually involved in cell fate regulation in physiological and tumorigenic processes [7]. In cancer cells, a wide range of glycosylation alterations have been observed [7,8]. These modifications influence tumour cell dissociation and invasion since glycans are mediators of cell–cell adhesion. One of the most important alterations is represented by an increased β1,6 branched **N-glycans**, associated with the increase in cell migration. In addition, the increase of sialylation of these branches has been observed to enhance cellular invasiveness. The **O-glycan** antigens truncated T, Tn, and sialyl T are considered tumour biomarkers since they are not normally expressed and appear early in tumorigenesis. In addition,

Highlights

Integrins have a well-recognized role in cancer, controlling cell identity, adhesion, migration, survival, and promoting metastasis.

Glycans are strongly involved in integrin stability and function.

Glycosylation of integrins and their interaction partners is dysregulated in tumorigenic processes.

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hyaluronic acid (HA) enrichment in the stroma is another glycan aberration occurring in malignancies [7]. Moreover, the alteration of integrin glycosylation has been reported to be one of the mechanisms of cell adhesion, migration, and survival in cancer [14,15,21]. Glycosylation of integrins is just one of the many aspects of the complicated network of interactions between integrins and the surrounding glycan species. In fact, integrin functions are also influenced by the glycosylation of the ligands, the interaction with ECM proteins, GAGS, and the composition of the cancer glycocalyx [9–12]. This review summarizes the complexity of the glycan–integrin regulation system, focusing on how the glycosylation of integrins influences cancer cell adhesion, migration, proliferation, and metastasis. Furthermore, we discuss how the alteration of the glyco-microenvironment can influence integrin function and focus in particular on the effect of the different glyco-species actively involved in tumour progression.

N-Glycosylation of Integrins

N-glycans are sugars that are covalently attached to proteins at asparagine (Asn) amino acids, and are classified into three types: oligomannose, complex, and hybrid (Figure 2). The roles of integrin N-glycosylation in cancer have been documented in several cell types [13–16]. Integrins contain more than 20 potential glycosylation sites and are the major N-glycan carriers [14]. The presence of the N-glycan core structure is essential for integrin heterodimerization, stabilization of conformation, expression at the cell membrane, and interaction with ligands [14–20] (Figure 3). The functional roles of core N-glycosylation are outlined in Table 1. N-glycosylation is dynamic and the remodelling of the N-glycan moieties occurring due to the action of glycotransferases regulates integrin binding to the substrates and has a key role in cell adhesion and migration [14,21] (Figure 3). The main N-glycosylation modifications that correlate with integrin-mediated malignant cell behaviour are β1,6-N-acetylglucosamine (β1,6-GlcNAc) branched structures, and sialylated and fucosylated structures [22–41,43,44,46].

Some cancer features are associated with N-glycans containing β1,6-GlcNAc branched structures, catalysed by N-acetylglucosaminyltransferase V (GnT-V). By contrast, N-acetylglucosaminyltransferase III (GnT-III) has been found to play an important role in the suppression of metastasis [43,44]. Specifically, GnT-III catalyses the addition of GlcNAc to a β1,4-mannose of the N-glycan core to form a 'bisecting' GlcNAc linkage. The introduction of a bisecting GlcNAc inhibits β1,6-GlcNAc branching formation, catalysed by GnT-V, which is closely associated with cancer metastasis, since GnT-V cannot use the bisected oligosaccharide as an acceptor substrate. The expression of GnT-V is elevated in multiple types of tumours, and their cognate sugars are correlated with metastasis and adverse patient prognosis. Therefore, GnT-V and GnT-III regulate cell migration, cell invasion, and metastasis via opposite mechanisms.

Branched β1,6-GlcNAc structures and bisecting GlcNAc have been observed on integrins α3β1 and α5β1, where they exhibit the same contrasting effects on cell adhesion, migration, and metastasis [13,22]. The overexpression of GnT-III and the resulting high levels of bisecting GlcNAc on the α5 subunit decrease binding affinity to fibronectin and inhibit spreading and migration of human fibrosarcoma and leukaemia cells [43]. A similar effect has been observed in gastric cancer cells, where GnT-III competes with GnT-V for the modification of the α3 subunit, causing a decrease in β1,6-GlcNAc branched N-glycans and impairing cell migration [23]. Consistently, GnT-III knockdown in a neuroblastoma cell line has resulted in increased migration with a concomitant increase in β1,6-GlcNAc branched N-glycans on the α3 subunit, confirming that GnT-V and GnT-III affect cell migration and invasion in an opposite way.

Furthermore, the overexpression of GnT-V promotes integrin α5β1-mediated migration of mouse leukaemia cells [24], while the knockdown of the GnT-V gene in mice reduces the
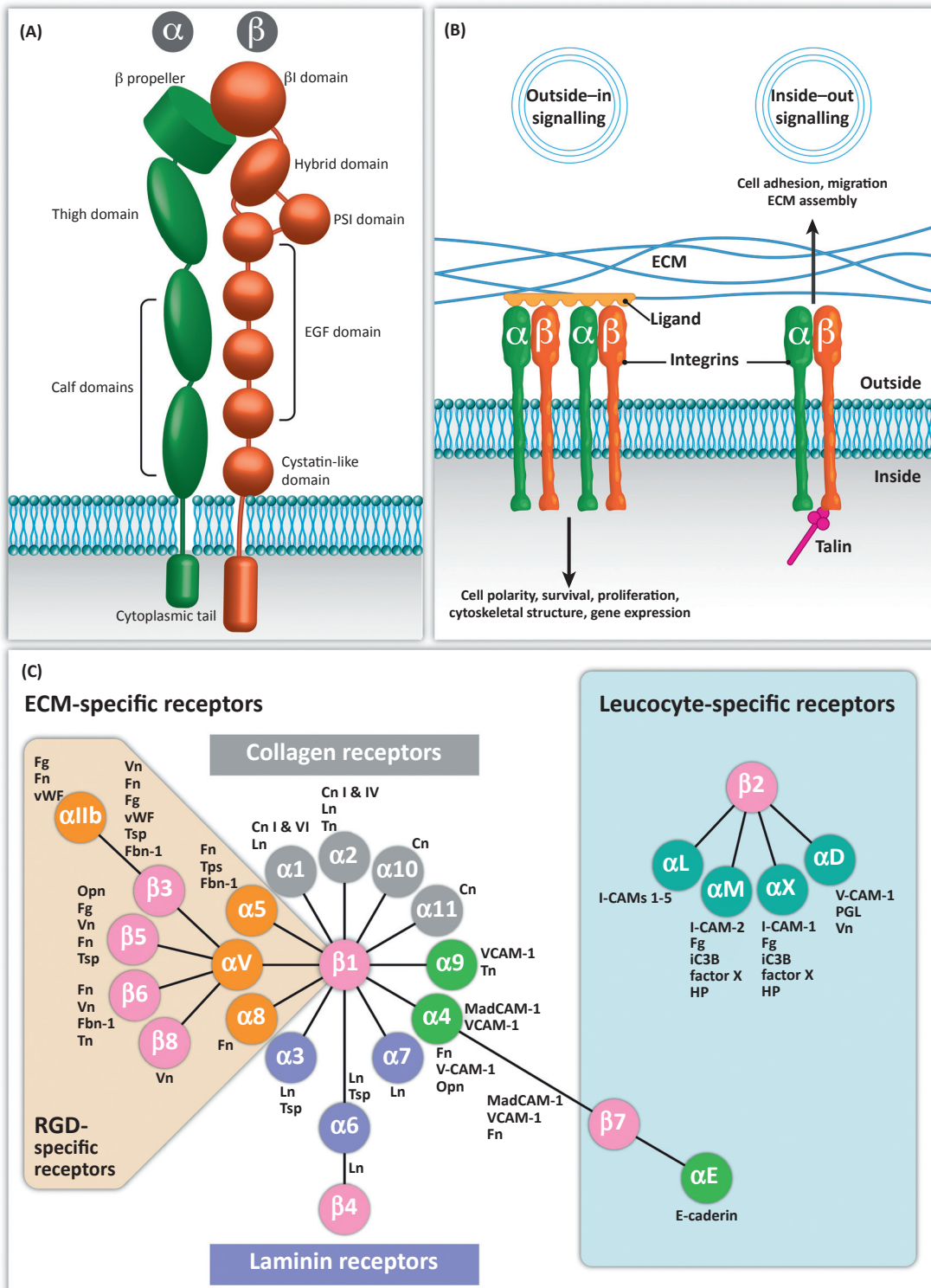
formation of mammary tumour growth and metastasis [25]. The transition of the metastatic state in melanoma cells is characterized by the enhanced expression of GnT-V and β1, 6-GlcNAc branched glycans on α3β1. In addition, in melanoma cells the transition to the metastatic state is characterized by increased β1,6-GlcNAc on α3β1 and the enhanced expression level of GnT-V [26,44]. Taken together, this evidence suggests that the competitive action of the enzymes GnT-III and GnT-V can remodel N-glycans on integrins α3β1 and α5β1, and that these modifications have an important role in regulating cancer cell migration during metastasis. The mechanism by which the β1,6-GlcNAc branched structure on integrins can mediate adhesion and migration of cancer cells is still unclear. It has been shown that complex N-glycans on the lower leg domains of the α5β1 integrin can allosterically regulate the affinity for the ligand, favouring the extended-open conformation of the ectodomain [27]. This suggests that large branched β1,6-GlcNAc N-glycans can maintain an active conformation of integrins and, therefore, can promote adhesion and migration of cancer cells.

Terminal sialylation of the β1 integrin is another N-glycan chain modification that mediates cancer cell adhesion [28–41]. N-sialylation occurs when sialyltransferases transfer sialic acid onto galactose residues previously added by galactose-1-phosphate uridylyltransferase (GALT) on the N-glycan core structure (Figure 3). An increase in sialylation, due to altered glycosyltransferases expression, has been associated with several cancers [7]. For example, α2,6-sialylation, which is catalysed by β-galactoside α2,6-sialyltransferase I (ST6Gal-I) – an enzyme with an altered expression in various tumours (stomach, colon, and ovarian cancer) – has been reported to be a predictive marker of a negative prognosis in colon cancer [7,30]. One of the mechanisms involved in cancer progression is likely to be α2,6-sialylation of integrins since several studies have shown that α2,6-sialic acid can influence integrin-mediated adhesion and signalling [28–37]. In breast cancer and hepatocarcinoma cells, α2,6 sialylation increases the binding of the β1 integrin with collagen I, laminin, and fibronectin, and consequently influenced their metastatic potential [28,29]. In addition, ST6Gal-I is overexpressed in colon adenocarcinoma, and the enzyme activity correlates with tumour cell invasiveness [31]. Several reports on ST6Gal-I indicate that hypersialylation of the β1 subunit of integrins is overexpressed in multiple established cancer cell lines (Table 1) [28–37]. Inducing the expression of the oncogene ras in colonocytes upregulated ST6Gal-I and consequently increased α2-6 sialylation of β1 integrins, and furthermore, hypersialylated β1 integrin has been found in colon carcinoma biopsies [31,32]. These findings suggest that the effects of altered sialylation in tumours are dependent on β1 integrin heterodimers. A similar behaviour has been observed in human colon cancer cells where the stable expression of ST6Gal-I resulted in an increased number of α2,6 sialylated β1 integrins, increased affinity to collagen and laminin, and consequently enhanced migration [40]. Furthermore, the knockdown of ST6Gal-I induced apoptosis and increased the sensitivity of cervical cancer cells to the chemotherapeutic cisplatin [36]. Another type of sialylation, involving α2,8-oligosialic acid, has been found on the α5 subunit of human melanoma and erythroleukaemia cells and has an effect on integrin function. The enzymatic removal of α2,8-polysialic acids from the α5 subunit inhibited melanoma cell adhesion to fibronectin, indicating that the polysialic acid on the α5 subunit of integrin α5β1 plays an important role in cell adhesion [37].

Overall, these studies suggest that the sialylation patterns can modulate the cellular interactions of integrin receptors with ECM ligands in several cancers. Altered sialylation causes significant conformational changes in key functional sites of both the β1 I-like domain and fibronectin, directly affecting the allosteric regulation of the binding [35]. Another proposed mechanism involves the galectin-mediated signalling. Galectins are a class of proteins that bind N-acetyllactosamine (Galβ1-3GlcNAc or Galβ1-4GlcNAc), and specifically galectin-3 is involved

acetylgalactosamine (GalNAc) and in some cases through mannose (Man) and fucose (Fuc).
Proteoglycans: a class of glycoproteins carrying GAGs linked through a covalent bond to threonine/serine.

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(A)

α β

β propeller
βI domain
Thigh domain
Hybrid domain
PSI domain
EGF domain
Calf domains
Cystatin-like domain
Cytoplasmic tail

(B)

Outside–in signalling
Inside–out signalling

Cell adhesion, migration ECM assembly

ECM
Ligand
Integrins
Outside
Inside
Talin

Cell polarity, survival, proliferation, cytoskeletal structure, gene expression

(C)

ECM-specific receptors

Leucocyte-specific receptors

Collagen receptors

Fg
Fn
vWF

Vn
Fn
Fg
vWF
Tsp
Fbn-1

Cn I & IV
Ln
Tn

αIIb

Cn I & VI
Ln

Cn

Fn
Tps
Fbn-1

α1 α2 α10

Opn
Fg
Vn
Fn
Tsp

β3

α5

α11

Cn

β5

αV

β1

α9 VCAM-1
Tn

Fn
Vn
Fbn-1
Tn

β6

α8

α4 MadCAM-1
VCAM-1

β8

Fn

α3 α7

Fn
V-CAM-1
Opn

Vn

Ln
Tsp

Ln

RGD-specific receptors

α6

MadCAM-1
VCAM-1
Fn

Ln

β4

Laminin receptors

β2

αL

αM αX

αD

I-CAMs 1-5

I-CAM-2
Fg
iC3B
factor X
HP

I-CAM-1
Fg
iC3B
factor X
HP

V-CAM-1
PGL
Vn

β7

αE

E-caderin

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in the apoptotic mechanisms [42]. When the β1 subunit is hypersialylated, the sialic acid might mask the underlying N-acetyllactosamine substrates and prevent the interaction with Gal-3 [31,33].

Furthermore, both sialylation and core fucosylation play a role in modulating integrin functions during cancer. Core fucosylation consists in the addition of α1,6-fucose to the core GlcNAc residue of N-glycans through the action of Fut8 [45]. Core fucosylation is a crucial feature in liver, lung, and breast cancers [7]. In liver cancer cells, particularly, the inhibition of the fucosylation pathway decreases the fucosylation levels of integrin β1 and consequently suppresses the downstream signalling, reducing tumour formation [46].

In conclusion, these studies indicate that N-glycan alterations observed on integrin subunits can influence integrin affinity for the ligands, and therefore regulate cell function towards a malignant phenotype. Targeting branched 1,6-GlcNAc structures, sialic acid, and fucose, as well as their related enzymes, in conjunction with integrin inhibition, represents a promising therapeutic approach.

O-Glycosylation of Integrins

O-glycans are covalent modifications of serine and threonine residues occurring on mammalian glycoproteins (Figure 2). Integrins are also O-glycosylated which influences the adhesion and migration of tumour cells; however, isolating O-glycans remains a significant technical challenge [47]. O-glycans are implicated in tumour progression and their roles differ depending on their structure: core 1 O-glycans have a procancer effect, while core 3 O-glycans have an anticancer effect (Figures 2 and 3) [48–53]. Core 1 O-glycosylation of the β1 subunit modulates its function in cell adhesion and migration [48]. The enzyme responsible for the synthesis of core 1 O-glycans is β1,3-galactosyltransferase (C1GALT1), which is overexpressed in hepatocellular carcinoma and is associated with metastasis and an adverse prognosis, suggesting that it is crucial in mediating cellular invasiveness [7,48]. The C1GALT1-mediated tumour effect is dependent on integrin β1, since when integrin β1 was blocked, in C1GALT1 overexpressing hepatocarcinoma cells, the suppression of adhesion, migration, and invasion was observed [48].

While core 1 O-glycosylation of β1 integrin promotes a malignant phenotype, core 3 O-glycosylation, in contrast, plays an opposite role. Core 3-O-glycans are downregulated in cancer because of the loss of expression of β3-N-acetylglucosaminyltransferase-6 (core 3 synthase), suggesting that this glycan modification is crucial in suppressing tumour formation and metastasis [51,52]. Specifically, when the expression of core 3 is restored in cancer pancreatic cells, integrin α2β1 expression is reduced, and focal adhesions and lamellipodia are

Figure 1. Overview of Integrin Structure, Signalling, and Specificity. (A) Integrins are membrane receptors consisting of an α- and a β-subunit. Each subunit has a large extracellular domain which binds ligands, a single transmembrane helix, and a short cytoplasmic portion. Both α- and β-subunits consist of different subdomains. Both α- and β-subunits present different structural domains. The α-chain is composed of four or five head domains a folded seven-bladed β-propeller domain, a thigh, and two calf domains. Nine of the 18 α-isoforms also present an additional immunoglobulin (I)-like domain inserted into the β-propeller domain (not shown in the figure). The β-subunit consists of a βI-like domain, a PSI (plexin/semaphoring/integrin) domain, a hybrid domain, four epidermal growth factor (EGF) repeats, and a membrane proximal-β tail (βTD). (B) Bidirectional integrin signalling: integrins bind ligands in the extracellular space, triggering an 'outside–in' signalling that controls cell polarity, cytoskeletal structure, gene expression, cell survival, and proliferation. In addition, they can mediate an 'inside–out' signalling triggered by an intracellular activator such as talin that binds to the β-integrin tail. This results in increased affinity for extracellular matrix (ECM), cell migration, and ECM remodelling and assembly. (C) Schematic representation of integrin specificity: in mammals, 18α- and 8β-integrin isoforms combine into 24 αβ receptors. These can be grouped based on their preferred ligand or can be grouped into ECM-binding receptors and leukocyte-binding receptors. The ECM-binding receptors can be subcategorized into collagen, fibronectin, and RGD-binding receptors. Every single receptor has the capability to bind other cellular or non-cellular molecules. Cn, collagen; Fbn-1, fibrillin 1; Fg, fibrinogen; Fn, fibronectin; HP, heparin; iC3b, a proteolytically inactive product of the complement fragment C3b; ICAM-I, intercellular adhesion molecule 1; Ln, laminin; MadCAM-1, mucosal vascular address in cell adhesion molecule 1; Opn, osteopontin; Tn, tenascin; Tsp, thrombospondin; VCAM-1, vascular cell adhesion molecule 1; Vn, vitronectin; vWF, von Willebrand factor.

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Box 1. The Role of Integrins in Cancer

- Integrins regulate cancer cell migration, invasion, survival, and promote tumour progression and metastasis [4,81].
- Integrins are present in cancer-associated stromal cells such as endothelial cells, bone marrow mesenchymal stem cells, immune cells, adipocytes, and fibroblasts where they regulate signalling towards cancer progression.
- In several malignancies, the expression of integrins αvβ3, αvβ5, α5β1, α6β4, α4β1, and αvβ6, in patient biopsies is associated with increased disease progression and a negative prognosis [4,82].
- Integrins are involved in cancer initiation and transformation by regulating the progenitor cell phenotypes and by cooperating in signalling with the oncogenes [4,81].
- Integrins synergistically interact with growth factors and cytokine receptors, mediating aspects of cancer progression including migration, invasion, and survival.
- Integrins have a role in cancer angiogenesis: they regulate the migration and the proliferation of endothelial cells and their interaction with pericytes is crucial for the formation of the new blood vessels that transport oxygen to tumour.
- Cancer metabolism, which presents some alterations, has an impact on integrin functions, modulating integrin transcription, membrane transport, and degradation. In particular, hypoxia reduces extracellular pH, aerobic glycolysis, and enhances glucose utilization, which is crucial in regulating integrins expression [83].
- The alteration of glycan structures and function observed in cancer has an impact in regulating integrin-mediated signalling.
- Integrin–ECM interactions can dictate drug resistance, through favouring a prosurvival and antiapoptotic status [81].
- Therapeutic strategies that aim to disrupt integrin function, ranging from synthetic peptides to humanized antibodies, have been reported [82]. Despite the promising *in vitro* and preclinical results, several limitations in efficacy have been observed in late-phase clinical trials [84].

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formed, resulting in the suppression of cell attachment and migration [51]. Core 3 regulates the heterodimerization of α2β1 integrin and cell surface expression. In this way, core 3 O-glycosylation allosterically prevents the subunit association of α2β1 integrin, reducing cancer formation and cell differentiation [52,53].

Another integrin O-glycosylation event that has been well characterized is O-sialylation. Specifically, the sialyl-Tn (STn) antigen, which originates from a premature stop in protein O-glycosylation, has been characterized for its role in tumour promotion and invasion and immune escape [7]. In bladder tumour cells, the expression of STn on integrins and the increased cell migration and invasion are a consequence of the action of the hypoxia-inducible factor-1α (HIF-1α; Figure 3) [54]. Changes in the O-sialylation of integrins also modulate another important cancer process, the epithelial–mesenchymal transition (EMT), which is characterized by changes in cell morphology, adhesiveness, and motility that results in metastasis. Indeed, in human keratinocytes it was observed that sialylation of integrin β4 was downregulated during EMT but was upregulated in the mesenchymal state after EMT. This suggests that changes in O-sialylation may be responsible for regulating β4-mediated adhesion during EMT [55]. These findings comprehensively indicate that targeting core 1, core 3, and O-sialyl-expressing integrins offers the potential to suppress different aspects of the underlying cancer mechanisms.

Integrin Interaction with the Surrounding Glyco-Microenvironment: The ECM–Glycocalyx Interface

The interaction of integrins with glycans present at the ECM–cell membrane interface also affects integrin function. Both N-glycosylation and O-glycosylation of integrin ligands in the matrix are crucial in mediating the binding of the ECM to integrins and the activation of signalling cascades in cancer progression [9,10,22,56,57]. Glycosylation of integrin substrates also influences the binding and activation of integrins. Glycosylation of ECM proteins such as collagens, laminins, and fibronectins, and integrin interaction with GAGs and other glycans of the glycocalyx [**glycosphingolipids (GSLs)** and other glycoproteins] can modulate integrins during cancer progression (Figure 4).
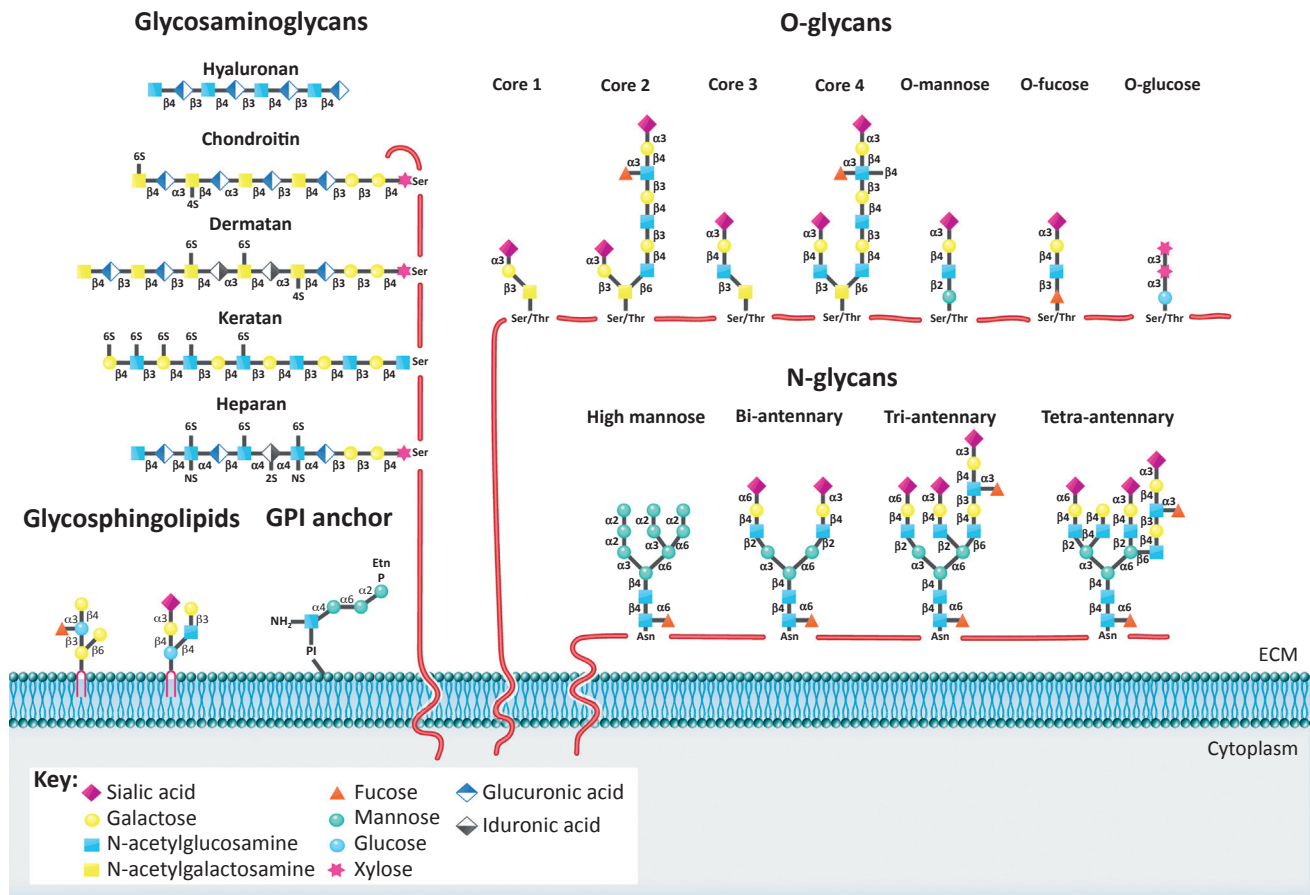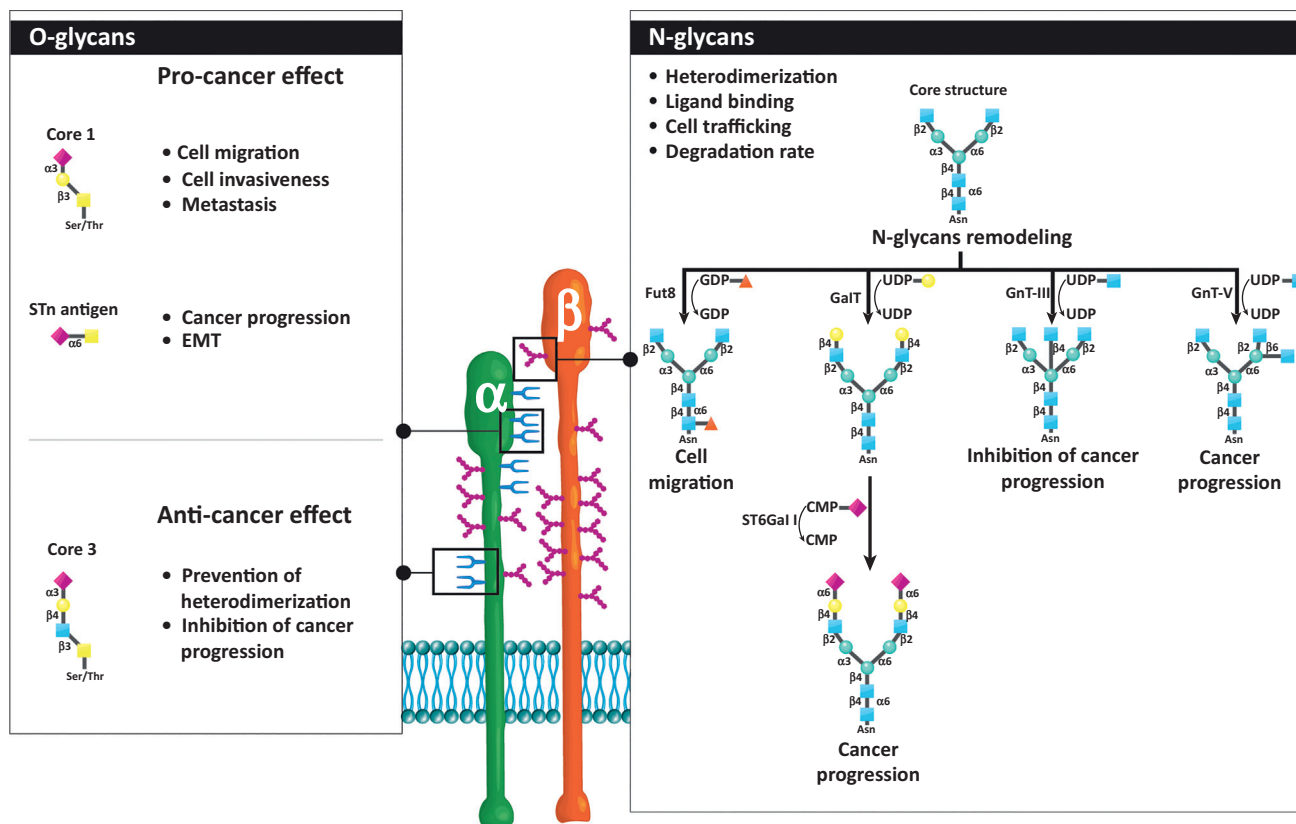
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Figure 2. Mammalian Glycans on the Cell Membrane. The main classes of glycans, glycosaminoglycans (GAGs), N-glycans, O-glycans, glycosphingolipids, and glycosylphosphatidylinositol (GPI) anchor. GAGs, heparin sulfate, chondroitin sulfate, hyaluronic acid, dermatan sulfate, and keratin sulfate, are depicted. NS, 2S, 4S, and 6S represent the sulfation positions on the GAGs chains. Representative examples of complex-type N (bi–tri–tetra–antennary) and high-mannose N-glycans are illustrated. Also depicted are core 1–4 O-glycans, O-mannose, O-fucose, and O-glucose structures. Glycan linkages are identified by the anomeric configuration (α or β) of the donor saccharide and by the ring position (1–6) of the acceptor sugar. The GPI anchor and examples of glycosphingolipids are also represented. Asn, asparagine; ECM, extracellular matrix; Etn–P, a phosphoethanolamine; PI, phosphatidylinositol; Ser, serine; Thr, threonine.

N-Glycosylation of ECM Components

N-glycosylation of collagens and laminins influences the binding to integrin receptors (Figure 4B) and promotes cancer cell adhesion [9,10]. Type IV collagen N-glycosylation effects on integrin binding have been documented in melanoma where cell adhesion was determined by the interaction between glycosylated collagen IV and α3β1 and α2β1 integrins [9]. In gastric cancer, laminin 332 is glycosylated with bisecting GlcNAc, which interacts with α3β1 integrin resulting in an increase in cell migration [10]. These data suggest that N-glycosylation of ECM proteins can regulate integrin affinity for the ligand, influence clustering, and thus resulting in cancer progression. Further studies are required to examine the nature of this interaction to validate whether targeting the N-glycosylated ECM proteins will represent a promising therapeutic approach.

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Box 2. Structural and Functional Complexity of Glycans

Glycosylation is a complex post-translational modification that allows the attachment of glycans to proteins, lipids, and other saccharides, and regulates their function [6,8]. The mammalian glycome has a wide structural heterogeneity and variety that results from combinatorial expression of more than 200 glycosyltransferases and glycosidases involved in synthesis and remodelling [8]. Biosynthesis is not template driven but regulated by many factors including the availability of nucleotide donors and the expression of enzymes [85]. Structural diversity of glycans is due to the number and sequences of monosaccharides units and to anomeric configuration, position, and differential branching of mono-saccharides. Figure 2 illustrates the main glycan categories: N-glycans, O-glycans, GAGs, GSLs, and **glycosylpho-sphatidylinositol anchor**. O-glycans and N-glycans are branched and/or linear structures that mainly enrich the glycocalyx [6,8]. By contrast, GAGs are linear structures, sulfated, negatively charged polysaccharides that enrich the ECM. In addition, glycan chains have other common modifications: derivatization of hydroxyls or amino groups, acylation, sulfation, methylation, and phosphorylation [85]. The glycome is dynamic and changes in response to intracellular and extracellular signals [7]. Glycosylation is crucial in determining the binding affinity for antigens, for cell surface proteins/receptors of other cells, for ECM proteins, and other soluble molecules, and mediates cell–cell interactions and ECM–cell crosstalk [15,21,86].

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Figure 3. N- and O-Glycosylation of Integrins and Associated Functions. Core 1 O-glycosylation and sialyl-Tn (STn) antigen on integrins are associated with cancer progression, while core-3 glycosylation prevents the dimerization of α and β chains. N-glycan core structure is involved in heterodimerization, ligand binding, cell trafficking, and the degradation rate of integrins. Glycan remodelling occurs through glycosylation reactions by glycosyltransferase. Remodelled N-glycans regulate cell adhesion and migration and consequently cancer progression. Enhanced expression of GnT-V results in an increase in integrin-mediated cell migration. By contrast, overexpression of GnT-III downregulates integrin-mediated cell migration. ST6Gal-I overexpression is associated with cancer progression, while Fut8 has a role in cell migration. Fut 8, α1,6-fucosyltransferase; GalT, hydroxyproline-O-galactosyltransferase; GnT-III, β1,4-N-acetylglucosaminyltransferase III; GnT-V, β1, 6 N-acetylglu-cosaminyltransferase V; ST6Gal-I, ST6 β-galactoside α2,6-sialyltransferase I.

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Table 1. Integrin Glycosylation: This Table Summarizes the Glycan Species Present on Integrin Structures and Their Associated Functional Role

| Integrin	Glycosylation	Function associated with glycosylation	System	Carrying-glycans domain	Refs
α5β1	N-glycans core structure	Association of α and β subunits	K562 leukaemia cells		[17]
		Transport on cell membrane			
		Presence of β1 subunits on the membrane	H7721 hepatocarcinoma cells	β1	[20]
		Active α5β1 expression and internalization	Breast cancer cells	β-Propeller	[14]
		Cell spreading and migration			
		Laminin interaction	HeLa cervical cancer cells	Calf1-2	[16,19]
		EGFR complex formation			
	Bisecting GlcNAc	Decreased binding affinity to fibronectin	B16-hm highly metastatic melanoma cells		[44]
		Inhibition of cell spreading and migration			
		Suppression of metastasis			
	Branched β1,6-GlcNAc	Metastasis potential	MT1, MTAg, and MTPy leukaemia cells		[24]
		Migration on fibronectin	Uveal WM1205Lu melanoma cells		[26]
		Suppression of metastasis	GnT-V$^{-/-}$ mice		[25]
	Terminal α2,6 hypersialylation	Collagen-I and fibronectin binding	HD3 colonocyte	β1	[40]
		Cell motility	SW48 colon epithelial cells		[31]
		Cancer progression	Human colon adenocarcinoma	β1	
	α2,8-sialylation	Cell adhesion to fibronectin	Human melanoma cell line G361	α5	[37]
	Poly-N-acetyllactosamine	Suppress the activation of β1 integrins	HCT116, SW480, SW620, Colo205, and HT29	β1	[87]
		β1 increased expression delayed degradation	Neuroblastoma cells	β1	[88]
		FK phosphorylation			
		Migration, invasion, tumour growth			
α2β1	α2,3 sialylation	Interaction and adhesion to Cn-I and AsGM1	C4-2B prostate cancer cells	α2	[41]
		Metastasis formation			
	O-Glycan Core 1	Enhanced invasiveness	Hepatocarcinoma cells SK-Hepl, HepG2, HA22T, and HCC36	β1	[48]
	O-Glycan Core 3	Prevented heterodimerization	Prostate carcinoma PC3	β1	[52,53]
		Inhibition of tumour formation and metastasis Inhibition	Gastrointestinal LNCaP		
α3β1	Bisecting GlcNAc	Reduced migration	MKN45 cells		[23]
	Branched β1,6-GlcNAc	Enhanced cell adhesion	WM1205Lu		[26]
		Increased metastatic potential	Melanoma cells		
		Migration on fibronectin			
		Association with CD151 tetraspanin	B16BL6 cells		[13]

Table 1. (continued)

Integrin	Glycosylation	Function associated with glycosylation	System	Carrying-gly-cans domain	Refs
		Cell spreading and motility			
	Core fucosylation	Cell migration and signalling	Liver cancer HepG2 cell	β1	[46]
	α2,6-linked sialic acid				
	Tri- and tetra-antennary β1,6-Galβ1-4Glc	Binding to vitronectin	Melanoma cells		[38]
αvβ3	Tri- and tetra-antennary β1,6-Galβ1-4Glc	Migratory ability	WM9 cells		[38]
	α2,6 and α2,3 sialic acid	Binding to vitronectin			
	High-level bisecting GlcNAc	Migratory capacity	WM239 cells		[38]
	High-mannose				
	α2,6-Linked sialic acid				
	Lower-level bisecting GlcNAc	Migratory capacity	WM793 cells		[39]
	High mannose		WM1205Lu cells		
	α2,3-Linked sialic acid				
α4β1	Terminal sialylation	Fibronectin binding	Human Burkitt's lymphoma β1 HBL-8 cells		[34]
	β-galactose	ECM invasion			[34]
		Increased motility			
		Metastasis			
α6β1	β 1,6-branched oligosaccharides	Impaired integrin presence on the membrane	NIH 3T3 fibroblasts	α6	[89]
		Impaired binding	Murine melanoma B16-F10 cells		[90]
α6β4	Branched β 1,6 GlcNAc	Modulation of adhesion and motility	Gastric cancer MKN45 cells	β4	[22]
	O- sialylation	Binding to laminin β4 phosphorylation, metastasis,	Human keratinocyte HaCaT cells		[55]
		EMT			

O-Glycosylation of ECM Components

O-glycosylation of fibronectin plays an important role during EMT that occurs in tumour progression and metastasis [56]. Prostate epithelial cells stimulated with tumour growth factor-β (TGF-β; inducer of EMT) show an upregulation of oncofoetal fibronectin (OFn). OFn presents O-glycosylation at the IIICS domain that is absent in the adult isoform but is crucial in the TGF-β-induced EMT process. This O-glycosylation induces a change from an epithelial to a fibroblastic morphology, enhances cell motility, decreases the expression of a typical epithelial cell marker, E-cadherin, and enhances expression of mesenchymal markers. It can be speculated that integrin–OFn interaction is involved in these cell changes as β5-integrins are crucial in mediating breast carcinoma cells adhesion during EMT [57]. Taken together, these results indicate that aberrant O-glycosylation of fibronectin has an impact on integrin-mediated signalling during cancer progression (Figure 4A). Although the molecular mechanisms triggered by this aberrant integrin–substrate interaction remain poorly understood, O-glycosylated OFn represents a promising target for anticancer drug development.

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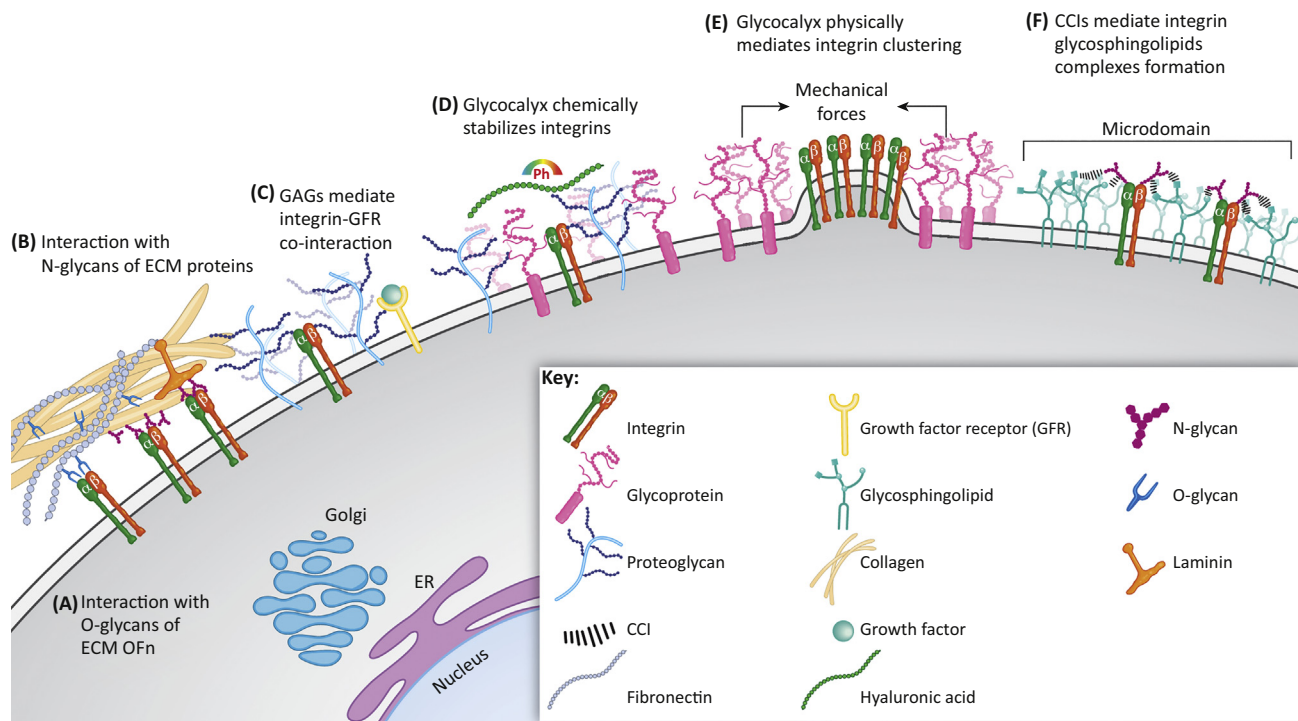
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Figure 4. Integrin–Glycans Interactions in Cancer in the Glyco-Microenvironment. (A) O-glycans of the aberrant oncofibronectin (OFn) interact with integrins mediating epithelial–mesenchymal transition. (B) N-glycans of extracellular matrix (ECM) major proteins, such as laminin and collagen, mediate the binding with integrin receptors. (C) The interaction between glycosaminoglycans (GAGs) and integrins activates integrins themselves and mediates their coupling with growth factor receptors (GFRs). (D) The glycocalyx preserves integrin conformation maintaining pH. (E) Mechanical action of the glycocalyx in mediating integrin clustering. (F) Carbohydrate–carbohydrate interactions (CCIs) between the glycans present on integrins and the glycosphingolipids mediate the formation of microdomains that are crucial in signal transduction. ER, endoplasmic reticulum.

GAGs and PGs interaction with integrins

Other relevant glycan-based molecules (GAGs and **proteoglycans**; Figure 2) that are found in the ECM or as membrane-anchored proteins in the glycocalyx regulate the function of integrins during cancer (Figure 4C). The main species involved in these interactions are hyaluronan, **heparin (HP)**, **heparan sulfate (HS)**, and **chondroitin sulfate (CS)** [11,58–72].

HA is implicated in integrin-mediated mechanotransduction in cell dysregulation including polarity, migration, differentiation, and survival [58,59]. HA is a major component of the ECM of the brain. Glioblastoma multiforme, a highly invasive brain tumour, is associated with an increase in HA secretion that leads to tissue stiffening, which directs cell migration, and drives cellular differentiation. The interaction of HA with CD44 receptors contributes to the mechanotransduction observed in glioblastoma multiforme and tumour cells, leading to improved adhesion and invasive migration [59].

HP is a GAG that exhibits antimetastatic activity. One of the mechanisms involved in the inhibition of cell–cell interactions is the regulation of integrin functions [60]. HP inhibits melanoma cell metastasis by blocking $\alpha 4\beta 1$ [very late antigen-4 (VLA-4)], which is important for metastatic progression, suggesting that VLA-4 is a potential therapeutic target [61].

Furthermore, HP can sequester the Cyr61 molecule, secreted in several malignancies that promote metastasis by activating α4β1-mediated signalling [62]. HS proteoglycans are also involved in the regulation of cancer cells by interacting with integrins [11]. Syndecans, in particular, interact with several integrins that mediate various aspects of tumorigenesis promoting tumour growth, cell migration, and metastasis [72,73]. Syndecan–integrin interactions also regulate the migration of endothelial cells that leads to the formation of new blood vessel in tumour angiogenesis. Among syndecans, syndecan-1, syndecan-2, and syndecan-4 have been studied in several cancer cell lines [63,65–70].

Syndecan-1 promotes growth, migration, and tumour angiogenesis, mediating the association of integrins with growth factor receptors (Figure 4C) via interaction of its extracellular domain [64,65]. Syndecan-1 binding with integrins αvβ3 and αvβ5 induces tumour cell spreading and invasion in human breast carcinoma cells [66]. In tumour angiogenesis, syndecan-1 also induces the clustering of integrins αvβ3 and αvβ5 and insulin-like growth factor receptor 1, leading to intracellular activation of the integrins by the cytoskeletal protein talin, promoting endothelial cell migration [64,67]. Overexpression of syndecan-1 in human mammary fibroblasts demonstrated an enhanced spindle-shaped morphology if plated in an over confluent state, with permissive conditions for ECM production. This event is partially reversed by siRNA-mediated silencing of β3 integrin expression. The interaction of syndecan-1 with integrins mediates ECM fibre alignment, a cancer phenomenon that directs the migration and invasiveness of breast carcinoma cells. Moreover, suppression of the αvβ3 integrin activity largely reversed the aligned ECM fibre architecture and consequently the invasive-permissive properties of the ECM induced by syndecan-1 [68]. HS chains and the ectodomain are necessary to drive the alignment of fibronectin fibres mediated by αvβ3 integrins of human mammary fibroblasts.

Recent studies indicate that the expression levels of syndecan-2 in breast cancer patients are associated with the metastatic ability of cells, the regulation of the morphology, and the invasion index [11,70]. Syndecan-2 regulates cancer activity in invasive lung cancer and colon cancer cells; specifically, its expression promotes E-cadherin shedding in colon cancer cells. In the case of metastatic colorectal cancer cells, syndecan-2 is overexpressed after its interaction with ECM components especially with fibronectin, produced by stromal fibroblasts. Furthermore, syndecan-1 and syndecan-4 also activate integrins α6β4 via the formation of a ternary complex integrin–syndecan–human epidermal growth factor receptor 2 that leads to tumour mammary carcinoma cells survival [65].

Another phenomenon observed in several mechanisms occurring in cancer progression that involves proteoglycans is the remodelling of their GAGs chains [71]. Structural modification of GAGs and their interaction with integrins is a key factor that drives the switch to the tumour phenotype. CS proteoglycans play a role in proliferation, migration, and metastasis and are emerging as relevant therapeutic targets [72]. CS proteoglycans associated with melanoma have shown the ability to interact with both α2β1 and α4β1 integrins, mediating cell migration and spreading. In melanoma, highly O-2-sulfated CS synergistically interacts with fibroblast growth factor 2 and potentiates integrin α5β1 signalling, mediating cell migration. Taken together, these findings indicate that synergistically targeting GAGs and integrins represents a promising therapeutic strategy to treat several aspects of tumorigenesis such as angiogenesis, invasion, and metastasis.

Glycocalyx: Mechanical and Chemical Regulation of Integrin Function

The glycocalyx is the set of proteoglycans, glycoproteins, GSLs, and soluble GAGs on the outer leaflet of the plasma membrane of eukaryotic cells. The glycocalyx is involved in cancer cell

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activities such as adhesion and spreading, influencing integrin interactions and functions [12,74,75]. Elevated expression of large glycan structures and glycoproteins is a universal feature of cancer cells. Aberrant glycosylation of glycocalyx components contributes to nearly every step in cancer proliferation, survival, angiogenesis, and metastatic dissemination [12,76–78]. The expression of the truncated O-glycan structures Tn and STn is correlated with poor survival in carcinoma patients. In addition, this immature O-glyco phenotype directly induces the oncogenic features in human keratinocytes, promoting cell growth and invasion by disrupting cell–cell adhesion contacts [78].

The glycocalyx plays a crucial role in maintaining a specific pH at the cell surface environment that protects cell receptor functions (Figure 4D). The disruption of the glycocalyx by chemical and enzymatic treatments impairs this pH and affects the integrin-mediated migration of melanoma cells [75]. The glycocalyx composition not only affects the chemical environment but also physically influences integrin-mediated cell adhesion [12,74]. Indeed, the glycocalyx mediates mechanotransduction and the flow-regulated invasion of metastatic cancer cells [74]. In particular, cell surface GAGs such as HS and HA on the cancer cell surface glycocalyx, together with integrin $\alpha3$, mediate migration and metastasis of human metastatic renal carcinoma cells. Furthermore, bulky glycoproteins, highly expressed in the cancer glycocalyx, promote the clustering of integrins at adhesion sites and alter the integrin state by applying tension to matrix-bound integrins [12]. This mechanical force guides integrins to assemble into mature adhesion complexes and plays a role in increasing growth factor signalling associated with metastasis (Figure 4E).

Another aspect of integrin–glycocalyx regulation in cancer is represented by carbohydrate–carbohydrate interactions (CCIs) [79,80]. Integrins are embedded in the cell membrane and surrounded by GSLs. GSLs including gangliosides interact with integrins, forming membrane microdomains resulting in the modulation of integrin-mediated activities. The formation of these dynamic microdomains is due to the establishment of CCIs between GSLs and glycans carried by integrins (Figure 4F). Terminal sialylation residues, in particular, are crucial in CCIs between GSLs and integrins [79]. Highly sialylated ganglioside GT1b interacts with high-mannose residues on the $\alpha5$ subunit of the $\alpha5\beta1$ integrin, regulating in this way $\alpha5\beta1$-mediated adhesion of epithelial cells to fibronectin [80]. GT1b also interacts with glycans present on integrin $\alpha2$, and modified sialylation is associated with tumour progression. $\alpha2,3$-Sialylation of $\alpha2$ subunits are required for the integrin $\alpha2\beta1$-dependent cell adhesion to collagen type I, and the same $\alpha2,3$-linked sialic acid residues on the integrin receptor are responsible for the interaction with the carbohydrate moiety of AsGM1, accounting for the complex formation between AsGM1 and $\alpha2\beta1$ integrin receptors [41].

Taken as a whole, this evidence reveals the complexity and the fine balance of the interaction between integrins and the glycocalyx, and the ability of the glycocalyx to influence integrin function. The mechanisms involved remain largely unexplored, and it is, as yet, very challenging to foresee the net effect of targeting the glycocalyx as therapeutic strategy.

Concluding Remarks

This review highlights the important aspects of glycosylation of integrins within the context of their function in cancer and underlines the primary role of the glyco-microenvironment in disease progression. Alterations of glycans regulate the function of integrins in several cancers cells including melanoma; breast, prostate, and gastrointestinal cancers; and hepatocarcinoma. Both glycosylation of integrin subunits and other glycan components present at the extracellular surface influence integrins' functions towards malignant cell behaviour. This

Outstanding Questions

Can a specific dysregulation of integrin glycosylation be considered as a biomarker in oncology? Can the specific alteration of glycoforms on integrins in tissues be used as a diagnostic tool?

Can the analysis of integrin glycosylation in serum or in biopsies be useful in determination of the prognosis for cancer patients?

What are the best models with which to study the impact of integrin glycosylation on cell–cell and cell–ECM events regulated by glycans?

How can we use tumour-associated integrin glycosylation to improve cancer therapy? Can we regulate and control integrin glycosylation and the glyco-microenvironment to modulate aggressive cell behaviour? Will the simultaneous targeting of glycan biosynthesis (glycosyltransferases) and blocking of specific integrins represent a novel therapeutic strategy for cancer patients? If yes, what will be the potential side effects?

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suggests that targeting specific glycans alterations can modulate integrin-mediated aggressive cells phenotypes, and therefore represent a potential therapeutic strategy. Both N-glycosylation and O-glycosylation of integrins are altered in several cancer cell lines, which directs cell fate towards a malignant phenotype. Further studies examining the integrin glycosylation of patient blood and biopsies are required to confirm whether alterations in the integrin glycosylation can serve as a prognosis predictor factor and a diagnostic tool for the detection of liver, stomach, colon, and ovarian cancers. However, due the dynamic changes in the glycosylation profile and also the dynamic remodelling of the extracellular milieu, novel analytical tools and methods are essential to elucidate not just the structural changes on glycosylated integrins, but also the causal link between integrin glycosylation and tumorigenesis. Interdisciplinary research efforts incorporating computational and analytical methods, new genetic tools for glycocalyx engineering, and three-dimensional *in vitro* models and assays that mimic the cell microenvironment are essential to clarify the mechanism of cell dysregulation in cancer progression. Targeting both the glycosyltransferases (gene knock down) and integrin functions (blocking antibody) simultaneously may represent a therapeutic strategy that needs to be investigated in preclinical models. One of the challenges is to elucidate the potential side effects caused by blocking integrin-related glycosylation. The development of experimental models that closely mimic the glyco-microenvironment is key to correlate the impact of glycosylation on integrin-mediated signalling in tumours with disease progression and prognosis to facilitate the translation of emerging therapeutic strategies to the clinical setting (see Outstanding Questions).

References

1. Barczyk, M. *et al.* (2010) Integrins. *Cell Tissue Res.* 339, 269–280
2. Hynes, R.O. (2002) Integrins: bidirectional, allosteric signaling machines. *Cell* 110, 673–687
3. Humphries, J.D. *et al.* (2006) Integrin ligands at a glance. *J. Cell Sci.* 119, 3901–3903
4. Desgrosellier, J.S. and Cheresh, D.A. (2010) Integrins in cancer: biological implications and therapeutic opportunities. *Nat. Rev. Cancer* 10, 9–22
5. Longmate, W. and DiPersio, C.M. (2017) Beyond adhesion: emerging roles for integrins in control of the tumor microenvironment. *F1000Research* 6, 1612
6. Moremen, K.W. *et al.* (2012) Vertebrate protein glycosylation: diversity, synthesis and function. *Nat. Rev. Mol. Cell Biol.* 13, 448–462
7. Pinho, S.S. and Reis, C.A. (2015) Glycosylation in cancer: mechanisms and clinical implications. *Nat. Rev. Cancer* 15, 540–555
8. Corfield, A.P. and Berry, M. (2015) Glycan variation and evolution in the eukaryotes. *Trends Biochem. Sci.* 40, 351–359
9. Stawikowski, M.J. *et al.* (2014) Glycosylation modulates melanoma cell alpha2beta1 and alpha3beta1 integrin interactions with type IV collagen. *J. Biol. Chem.* 289, 21591–21604
10. Kariya, Y. *et al.* (2008) N-glycosylation of laminin-332 regulates its biological functions. A novel function of the bisecting GlcNAc. *J. Biol. Chem.* 283, 33036–33045
11. Soares, M.A. *et al.* (2015) Heparan sulfate proteoglycans may promote or inhibit cancer progression by interacting with integrins and affecting cell migration. *Biomed Res. Int.* 2015, 453801
12. Paszek, M.J. *et al.* (2014) The cancer glycocalyx mechanically primes integrin-mediated growth and survival. *Nature* 511, 319–325
13. Ranjan, A. *et al.* (2014) Glycosylation of the laminin receptor (alpha3beta1) regulates its association with tetraspanin CD151: impact on cell spreading, motility, degradation and invasion of basement membrane by tumor cells. *Exp. Cell Res.* 322, 249–264
14. Gu, J. and Taniguchi, N. (2004) Regulation of integrin functions by N-glycans. *Glycoconj. J.* 21, 9–15
15. Gu, J. *et al.* (2009) Importance of N-glycosylation on alpha5beta1 integrin for its biological functions. *Biol. Pharm. Bull.* 32, 780–785
16. Hang, Q. *et al.* (2017) A key regulator of cell adhesion: identification and characterization of important N-Glycosylation sites on integrin alpha5 for cell migration. *Mol. Cell. Biol.* 37, e00558–16
17. Zheng, M. *et al.* (1994) Functional role of N-glycosylation in alpha 5 beta 1 integrin receptor. De-N-glycosylation induces dissociation or altered association of alpha 5 and beta 1 subunits and concomitant loss of fibronectin binding activity. *J. Biol. Chem.* 269, 12325–12331
18. Cai, X. *et al.* (2017) The importance of N-glycosylation on beta3 integrin ligand binding and conformational regulation. *Sci. Rep.* 7, 4656
19. Hang, Q. *et al.* (2015) Integrin alpha5 suppresses the phosphorylation of epidermal growth factor receptor and its cellular signaling of cell proliferation via N-glycosylation. *J. Biol. Chem.* 290, 29345–29360

Trends in Cancer

20. She, S. *et al.* (2010) Nm23-H1 suppresses hepatocarcinoma cell adhesion and migration on fibronectin by modulating glycosylation of integrin beta1. *J. Exp. Clin. Cancer Res.* 29, 93

21. Janik, M.E. *et al.* (2010) Cell migration-the role of integrin glycosylation. *Biochim. Biophys. Acta* 1800, 545–555

22. Kariya, Y. *et al.* (2010) Bisecting GlcNAc residues on laminin-332 down-regulate galectin-3-dependent keratinocyte motility. *J. Biol. Chem.* 285, 3330–3340

23. Zhao, Y. *et al.* (2006) N-acetylglucosaminyltransferase III antagonizes the effect of N-acetylglucosaminyltransferase V on alpha3-beta1 integrin-mediated cell migration. *J. Biol. Chem.* 281, 32122–32130

24. Asada, M. *et al.* (1997) Increased expression of highly branched N-glycans at cell surface is correlated with the malignant phenotypes of mouse tumor cells. *Cancer Res.* 57, 1073–1080

25. Granovsky, M. *et al.* (2000) Suppression of tumor growth and metastasis in Mgat5-deficient mice. *Nat. Med.* 6, 306–312

26. Pochec, E. *et al.* (2013) Expression of integrins alpha3beta1 and alpha5beta1 and GlcNAc beta1,6 glycan branching influences metastatic melanoma cell migration on fibronectin. *Eur. J. Cell Biol.* 92, 355–362

27. Li, J. *et al.* (2017) Conformational equilibria and intrinsic affinities define integrin activation. *EMBO J.* 36, 629–645

28. Yuan, Y. *et al.* (2016) Effect of alpha 2,6 sialylation on integrin-mediated adhesion of breast cancer cells to fibronectin and collagen IV. *Life Sci.* 149, 138–145

29. Yu, S. *et al.* (2013) Caveolin-1 up-regulates integrin alpha2,6-sialylation to promote integrin alpha5beta1-dependent hepatocarcinoma cell adhesion. *FEBS Lett.* 587, 782–787

30. Lu, J. and Gu, J. (2015) Significance of beta-galactoside alpha2,6 sialyltranferase 1 in cancers. *Molecules* 20, 7509–7527

31. Seales, E.C. *et al.* (2005) Hypersialylation of beta1 integrins, observed in colon adenocarcinoma, may contribute to cancer progression by up-regulating cell motility. *Cancer Res.* 65, 4645–4652

32. Shaikh, F.M. *et al.* (2008) Tumor cell migration and invasion are regulated by expression of variant integrin glycoforms. *Exp. Cell Res.* 314, 2941–2950

33. Zhuo, Y. and Bellis, S.L. (2011) Emerging role of alpha2,6-sialic acid as a negative regulator of galectin binding and function. *J. Biol. Chem.* 286, 5935–5941

34. Suzuki, O. *et al.* (2015) Sialylation and glycosylation modulate cell adhesion and invasion to extracellular matrix in human malignant lymphoma: dependency on integrin and the Rho GTPase family. *Int. J. Oncol.* 47, 2091–2099

35. Pan, D. and Song, Y. (2010) Role of altered sialylation of the I-like domain of beta1 integrin in the binding of fibronectin to beta1 integrin: thermodynamics and conformational analyses. *Biophys. J.* 99, 208–217

36. Zhang, X. *et al.* (2016) Knockdown of ST6Gal-I increases cisplatin sensitivity in cervical cancer cells. *BMC Cancer* 16, 949

37. Nadanaka, S. *et al.* (2001) Occurrence of oligosialic acids on integrin alpha 5 subunit and their involvement in cell adhesion to fibronectin. *J. Biol. Chem.* 276, 33657–33664

38. Kremser, M.E. *et al.* (2008) Characterisation of alpha3beta1 and alpha(v)beta3 integrin N-oligosaccharides in metastatic melanoma WM9 and WM239 cell lines. *Biochim. Biophys. Acta* 1780, 1421–1431

39. Pochec, E. *et al.* (2015) Aberrant glycosylation of alphavbeta3 integrin is associated with melanoma progression. *Anticancer Res.* 35, 2093–2103

40. Seales, E.C. *et al.* (2003) Ras oncogene directs expression of a differentially sialylated, functionally altered beta1 integrin. *Oncogene* 22, 7137–7145

41. Van Slambrouck, S. *et al.* (2014) Carbohydrate-to-carbohydrate interactions between alpha2,3-linked sialic acids on alpha2 integrin subunits and asialo-GM1 underlie the bone metastatic behaviour of LNCAP-derivative C4-2B prostate cancer cells. *Biosci. Rep.* 34, e00138

42. Nangia-Makker, P. *et al.* (2007) Galectin-3 in apoptosis, a novel therapeutic target. *J. Bioenerg. Biomembr.* 39, 79–84

43. Guo, H.B. *et al.* (2002) Aberrant N-glycosylation of beta1 integrin causes reduced alpha5beta1 integrin clustering and stimulates cell migration. *Cancer Res.* 62, 6837–6845

44. Yoshimura, M. *et al.* (1995) Suppression of lung metastasis of B16 mouse melanoma by N-acetylglucosaminyltransferase III gene transfection. *Proc. Natl. Acad. Sci. U. S. A.* 92, 8754–8758

45. Miyoshi, E. *et al.* (1999) The alpha1-6-fucosyltransferase gene and its biological significance. *Biochim. Biophys. Acta* 1473, 9–20

46. Zhou, Y. *et al.* (2017) Inhibition of fucosylation by 2-fluorofucose suppresses human liver cancer HepG2 cell proliferation and migration as well as tumor formation. *Sci. Rep.* 7, 11563

47. Jensen, P.H. *et al.* (2012) Structural analysis of N- and O-glycans released from glycoproteins. *Nat. Protoc.* 7, 1299–1310

48. Liu, C.H. *et al.* (2014) C1GALT1 promotes invasive phenotypes of hepatocellular carcinoma cells by modulating integrin beta1 glycosylation and activity. *PLoS One* 9, e94995

49. Gomes, J. *et al.* (2017) Early GalNAc O-glycosylation: pushing the tumor boundaries. *Cancer Cell* 32, 544–545

50. Tsuboi, S. *et al.* (2012) Two opposing roles of O-glycans in tumor metastasis. *Trends Mol. Med.* 18, 224–232

51. Radhakrishnan, P. *et al.* (2013) Expression of core 3 synthase in human pancreatic cancer cells suppresses tumor growth and metastasis. *Int. J. Cancer* 133, 2824–2833

52. Lee, S.H. and Fukuda, M. (2013) Study of the biological functions of mucin type core 3 O-glycans. *Methods Mol. Biol.* 1022, 41–50

53. Lee, S.H. *et al.* (2009) Core3 O-glycan synthase suppresses tumor formation and metastasis of prostate carcinoma PC3 and LNCaP cells through down-regulation of alpha2beta1 integrin complex. *J. Biol. Chem.* 284, 17157–17169

54. Peixoto, A. *et al.* (2016) Hypoxia enhances the malignant nature of bladder cancer cells and concomitantly antagonizes protein O-glycosylation extension. *Oncotarget* 7, 63138–63157

55. Du, J. *et al.* (2015) Dynamic sialylation in transforming growth factor-beta (TGF-beta)-induced epithelial to mesenchymal transition. *J. Biol. Chem.* 290, 12000–12013

56. Freire-de-Lima, L. *et al.* (2011) Involvement of O-glycosylation defining oncofetal fibronectin in epithelial-mesenchymal transition process. *Proc. Natl. Acad. Sci. U. S. A.* 108, 17690–17695

57. Bianchi, A. *et al.* (2010) Role of beta5-integrin in epithelial-mesenchymal transition in response to TGF-beta. *Cell Cycle* 9, 1647–1659

58. Chopra, A. *et al.* (2014) Augmentation of integrin-mediated mechanotransduction by hyaluronic acid. *Biomaterials* 35, 71–82

59. Kim, Y. and Kumar, S. (2014) CD44-mediated adhesion to hyaluronic acid contributes to mechanosensing and invasive motility. *Mol. Cancer Res.* 12, 1416–1429

60. Borsig, L. (2010) Heparin as an inhibitor of cancer progression. *Prog. Mol. Biol. Transl. Sci.* 93, 335–349

61. Schlesinger, M. *et al.* (2014) The role of VLA-4 binding for experimental melanoma metastasis and its inhibition by heparin. *Thromb. Res.* 133, 855–862

62. Schmitz, P. *et al.* (2013) Cyr61 is a target for heparin in reducing MV3 melanoma cell adhesion and migration via the integrin VLA-4. *Thromb. Haemost.* 110, 1046–1054

63. Rapraeger, A.C. (2013) Synstatin: a selective inhibitor of the syndecan-1-coupled IGF1R-alphavbeta3 integrin complex in tumorigenesis and angiogenesis. *FEBS J.* 280, 2207–2215

64. Rapraeger, A.C. *et al.* (2013) Vascular endothelial-cadherin stimulates syndecan-1-coupled insulin-like growth factor-1 receptor and cross-talk between alphaVbeta3 integrin and vascular endothelial growth factor receptor 2 at the onset of endothelial cell dissemination during angiogenesis. *FEBS J.* 280, 2194–2206

65. Wang, H. *et al.* (2014) Cytoplasmic domain interactions of syndecan-1 and syndecan-4 with alpha6beta4 integrin mediate human epidermal growth factor receptor (HER1 and HER2)-dependent motility and survival. *J. Biol. Chem.* 289, 30318–30332

Trends in Cancer

66. Beauvais, D.M. et al. (2004) The syndecan-1 ectodomain regulates alphavbeta3 integrin activity in human mammary carcinoma cells. J. Cell Biol. 167, 171–181

67. Beauvais, D.M. and Rapraeger, A.C. (2010) Syndecan-1 couples the insulin-like growth factor-1 receptor to inside-out integrin activation. J. Cell Sci. 123, 3796–3807

68. Yang, N. and Friedl, A. (2016) Syndecan-1-induced ECM fiber alignment requires integrin alphavbeta3 and syndecan-1 ectodomain and heparan sulfate chains. PLoS One 11, e0150132

70. Lim, H.C. and Couchman, J.R. (2014) Syndecan-2 regulation of morphology in breast carcinoma cells is dependent on RhoGTPases. Biochim. Biophys. Acta 1840, 2482–2490

71. Theocharis, A.D. and Karamanos, N.K. (2017) Proteoglycans remodeling in cancer: underlying molecular mechanisms. Matrix Biol. Published online November 8, 2017. http://dx.doi.org/10.1016/j.matbio.2017.10.008

72. Afratis, N. et al. (2012) Glycosaminoglycans: key players in cancer cell biology and treatment. FEBS J. 279, 1177–1197

73. Afratis, N.A. et al. (2017) Syndecans – key regulators of cell signaling and biological functions. FEBS J. 284, 27–41

74. Qazi, H. et al. (2013) Cancer cell glycocalyx mediates mechanotransduction and flow-regulated invasion. Integr. Biol. (Camb.) 5, 1334–1343

75. Krahling, H. et al. (2009) The glycocalyx maintains a cell surface pH nanoenvironment crucial for integrin-mediated migration of human melanoma cells. Pflugers Arch. 458, 1069–1083

76. Hauselmann, I. and Borsig, L. (2014) Altered tumor-cell glycosylation promotes metastasis. Front. Oncol. 4, 28

77. Croci, D.O. and Rabinovich, G.A. (2014) Linking tumor hypoxia with VEGFR2 signaling and compensatory angiogenesis: glycans make the difference. Oncoimmunology 3, e29380

78. Radhakrishnan, P. et al. (2014) Immature truncated O-glycophenotype of cancer directly induces oncogenic features. Proc. Natl. Acad. Sci. U. S. A. 111, E4066–E4075

79. Hakomori, S. (2004) Carbohydrate-to-carbohydrate interaction, through glycosynapse, as a basis of cell recognition and membrane organization. Glycoconj. J. 21, 125–137

80. Wang, X. et al. (2001) Carbohydrate-carbohydrate binding of ganglioside to integrin alpha(5) modulates alpha(5)beta(1) function. J. Biol. Chem. 276, 8436–8444

81. Seguin, L. et al. (2015) Integrins and cancer: regulators of cancer stemness, metastasis, and drug resistance. Trends Cell. Biol. 25, 234–240

82. Hamidi, H. et al. (2016) The complexity of integrins in cancer and new scopes for therapeutic targeting. Br. J. Cancer 115, 1017–1023

83. Ata, R. and Antonescu, C.N. (2017) Integrins and cell metabolism: an intimate relationship impacting cancer. Int. J. Mol. Sci. 18, E189

84. Raab-Westphal, S. et al. (2017) Integrins as therapeutic targets: successes and cancers. Cancers (Basel) 9, E110

85. Muthana, S.M. et al. (2012) Modifications of glycans: biological significance and therapeutic opportunities. ACS Chem. Biol. 7, 31–43

86. Varki, A. (1993) Biological roles of oligosaccharides: all of the theories are correct. Glycobiology 3, 97–130

87. Chen, C.H. et al. (2014) Beta-1,4-galactosyltransferase III suppresses beta1 integrin-mediated invasive phenotypes and negatively correlates with metastasis in colorectal cancer. Carcinogenesis 35, 1258–1266

88. Chang, H.H. et al. (2013) Beta-1,4-galactosyltransferase III enhances invasive phenotypes via beta1-integrin and predicts poor prognosis in neuroblastoma. Clin. Cancer Res. 19, 1705–1716

89. Jasiulionis, M.G. et al. (1996) Alpha6beta1-integrin, a major cell surface carrier of beta1-6-branched oligosaccharides, mediates migration of EJ-ras-transformed fibroblasts on laminin-1 independently of its glycosylation state. Cancer Res. 56, 1682–1689

90. Chammas, R. et al. (1993) Functionally distinct roles for glycosylation of alpha and beta integrin chains in cell-matrix interactions. Proc. Natl. Acad. Sci. U. S. A. 90, 1795–1799

91. Fässler, R. and Meyer, M. (1995) Consequences of lack of beta 1 integrin gene expression in mice. Genes Dev. 9, 1896–1908