Integrins and Sugars: Implications in Cancer

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Keywords: integrins, glycosylation, cancer, glyco-micronvirnment, interactions

Abstract

Integrins are transmembrane receptors able to coordinate ECM-cell and cell-cell interactions, signal transmission, gene expression and cell functionalities. The aberration of integrin function is one of the well recognised mechanisms of cancer. Today, it is well known that integrin activities are 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 variety in both structure and function. Widely expressed both in ECM and on cell surface, they also have a crucial role in mediating cell proliferation, survival, and metastasis during cancer. The purpose of this review is to provide an overview of how the glycoenvironment regulates integrin function influencing the cancerous process.

1. Integrins and glycans: important players in cancer

Integrins are major regulators of cellular events during development, normal homeostasis and diseases [1]. In 1995, Meyer and Foessler showed that β1 integrin knockout embryos were failing at the stage of blastocistis implantation, proving the essential role of these receptors [2]. As crucial mediators of cell interactions, integrins have also a well recognised role in cancer, controlling cell migration, survival, and promoting metastasis [3]. Integrin function is regulated by a wide variety of molecular events that includes glycosylation. The alteration of integrin glycosylation has been described as one of the mechanisms of cancer [4-7]. This, though, 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 influenced by the glycosylation of the ligands, the interactions with ECM proteins, glycosaminoglycans, and the composition of the glycocalyx [8-11]. Since there is now only poor knowledge of role of the latter events in cancer, with this review we aim to summarise the complexity of the glycan-integrin regulation system and consider future directions in the identification of clinical targets.

2. Glycomicroenvironment: effect on integrin structure and function

Integrins are a family of heterodimeric cell membrane receptors consisting of two non-covalently linked subunits, α and β **(Figure 1a)**. In mammals, 18α- and 8β- integrin isoforms combine into 24 αβ receptors[12]. Integrins are known for their ability to bind a wide variety of ligands: they recognize and bind several ECM components such as collagens, laminins and fibronectins; but they also mediate cell-cell interaction by binding other cell receptors or soluble molecules **(Figure 1c)**[13]**.** Integrins act as a bridge between the cell and the ECM in transducing bidirectional information **(Figure 1b)**. They represent the primary receptors on tumor and stromal cells and are involved in cell – microenvironment interactions, cell adhesion, migration and chemoresistance mechanisms [14].

All cells in nature are covered with a dense and large array of sugars and the ECM of eukaryotes is rich in glycan-based structures **(Figure 2)** [15]. Glycosylation is crucial in determining the binding affinity with antigens, cell surface proteins/receptors of other cells, ECM proteins and other soluble molecules, resulting in the mediation of cell-cell interactions and ECM-cell crosstalk. These events control a complexity of cellular activities such as cell adhesion, spreading and migration [5-7, 16]. The glycomicroenvironment involves several glycan based modifications, ranging from PTMs (N- and O-linked) and oligo- and polisaccharides of cell surface and ECM proteins. These glyco-components are mutually involved in cell fate regulation in physiological and tumorigenic processes[17].

2.1 N-glycosylation of integrins: structural and functional role in cancer

Integrins are major N-glycan carrier proteins since they contain more than 20 potential glycosylation sites [18]. The presence of N-glycan core structure is essential in, heterodimerisation, the stabilization of the integrin conformation and functional role as well as in their expression on the cell membrane, and in their interactions with ligands [5, 18-23]**(Figure 3).** The roles of integrin glycosylation in the latter activities have been documented in cancer progression [5, 24]. The functional roles of N-glycosylation are outlined in **Table 1**.

N-glycosylation is dynamic: the remodelling of the N-glycan moieties due to the action of the glycosyltranferases in response to other signal molecules regulates integrin binding to the substrate, playing a key role in cell adhesion and migration[6, 18] **(Figure 3)**. The major enzymes involved in N-glycan modification are N-acetylglycosaminyltransferase III (GnT III), N-acetylglycosaminyl transferase V (GnT-V) and α2,6 sialyltransferase (ST6GalI). GnT-III catalyses the addition of GlcNAc to β1, 4-linked N-acetylglucosamine to mannose, producing a bisecting GlcNAc linkage. This modification inhibits the additional elongation of N-glycans. GnT-V mediates the formation of β1, 6-GlcNAc branching structures and these do not proceed because the bisected N-glycans cannot be used as a substrate. The bisecting GlcNAc has cancer-suppressive potential and an anti-metastatic effect [25, 26]. The overexpression of GnT-III and the resulting high levels of bisecting GlcNac on the α5 subunit decreased binding affinity to fibronectin and inhibited cell spreading and migration [25, 26] . In contrast, overexpression of GnT-V promoted integrin α5β1-mediated cell migration on fibronectin [27]. Furthermore, increased activity of this enzyme was found in metastatic cell lines, and knockout of the GnT-V gene in mice inhibited the formation of metastasis [25, 28]. Several studies have shown the pro-cancer effect of the branched β1,6 GlcNAc on different integrins[29-34].

Glycan remodeling occurring through the action of the enzymes GnT-III and GnT-V has been found also to regulate the function of α3β1 [7, 33, 35].

GnT-III antagonises the effect of GnT-V, affecting α3β1 integrin-mediated cell migration[35]. GlcNAcβ1 N-glycosylation of α3β1 and the expression level of GnT-V increase during the transition to the metastatic stage in melanoma cells, highlighting their important role in the migration and aggressiveness of the tumor [36].

Another N-glycan chain modification that is crucial in mediating cell adhesion is the terminal sialylation of the β1 integrin [37-50]. N-sialylation occurs when sialyltransferases transfer sialic acid on galactose residues which are previously added by Galactose-1-phosphate uridylyltransferase (or GALT) on the N-glycan core structure **(Figure 3)**.

α2,6 sialylation, particularly has been shown to affect the binding of the β1 integrin with collagen I, laminin and fibronectin and consequently influences the adhesiveness and metastatic potential[37, 38]. α2,6 sialylation is considered a marker of cancer progression: the overexpression of the enzyme responsible for the addition of α2,6 to N-glycans, (ST6GalI), is associated with metastasis and poor survival; indeed, several types of tumour express high contents of α2, 6 sialylation [39]. Hypersialylation of β1 integrins has been observed in colon adenocarcinona and may be one of the causes of cancer progression [40, 41]. Examination of colon carcinoma biopsies revealed hypersialylated β1 integrin. The stable expression of ST6GalI (with α2-6 sialylatedβ1 integrins) determined an increased attachment to collagen-I and laminin and enhanced migration toward collagen in human colon epithelial cells, in contrast to cells with completely unsialylated β1 integrins. Zhuo and Bellis[42] observed that hypersialylated integrin β1 prevents the interaction with Gal-3, inhibiting Gal-3-mediated apoptosis and consequently promoting survival. Along with apoptosis inhibition, it was also observed that sialylation enhances the Rho GTPases family, resulting in increased invasiveness and a poor prognosis for malignant lymphoma patients [43]. Pan and Song [44] characterised the thermodynamics of the interaction between the altered sialylation of the β1 I-like domain and fibronectin. Altered sialylation caused significant conformational changes in key functional sites of both the β1 I-like domain and fibronectin, directly affecting the allosteric regulation of the binding. Furthermore, the knockdown of ST6GalI induces cell apoptosis, inhibits the invasiveness of cells and increases the sensitivity of cervical cancer cells to cisplatin [45].
Another type of sialylation, α2,8- oligosialic acid, has been found on the α5 subunit of cancer cells such as G361 human melanoma cells, chronic and erythroleukemia K562 cells. In addition, the enzymatic removal of α2, 8- polysialic acids from the α5 integrin subunit prevented melanoma cell adhesion to fibronectin, suggesting that the polysialic acid on the α5 subunit of integrin α5β1 plays an important role in cell adhesion to fibronectin [46]. Overall, these studies suggest that the sialylation patterns of both α and β subunits can modulate the cellular interactions of integrin receptors with ECM ligands.

Furthermore, core fucosylation also plays a role in modulating integrin functions. Core fucosyltransferase 8 (Fut8) is the enzyme involved in the formation of GlcNac bisected β1, 6-branched and core fucosylated structures [51]. Core fucosylation is a crucial feature of liver cancer: inhibition of the fucosylation pathway determined decreased fucosylation levels of integrin β1, suppressing the downstream signaling and reducing tumor formation, suggesting that core fucosylation is crucial in integrin-mediated cell proliferation and migration [52].

2.2 O-glycosylation of integrins and tumor progression

Fewer studies are available about the role of O-glycosylation of integrins due to the technical limitations and the difficulty of isolation [53]. Nevertheless, it has been recognised that O-glycans are also implicated in tumor progression, and their roles differ depending? upon their structure: Core 1 and Core 2 structures have a pro-cancer effect, while Core 3 has an anti-cancer effect **(Figure 3)** [54-59]. β1, 3-galactosyltransferase (C1GALT1), the enzyme responsible for the synthesis of the O-Glycan Core 1, was observed to modify O-glycans on integrin β1 and regulate integrin β1 activity as well as its downstream signaling. This event has been associated with enhancement of hepatocarcinoma cells invasiveness [54]. Furthermore, in this tumor, GALNT1 relocates from Golgi to the endoplasmic reticulum, where (it) increases the glycosylation metalloproteinase MMP14 that mediates the extracellular matrix-degradation, that favors cell migration [55].

Core 2 O-glycans permit tumor cells to escape natural killer (NK) cells of the immune system and prolong their lives in the circulation, promoting cancer metastasis[56]. Core 3 O-glycans inhibit tumor formation and metastasis by modulating integrin-mediated signaling [57, 58]. Specifically, Core 3 O-glycan can reduce integrin α2β1 expression, that leads to disactivation of focal adhesion kinase and consequently altered cell lamellipodia formation [57].

Furthermore, Core 3 O-glycan prevents the heterodimerisation of α2β1 integrin, resulting in the suppression of prostate cancer formation and gastrointestinal cell differentiation [58, 59].

More recently, it has also been reported that the tumor hypoxic environment can influence the expression of O-glycans. In bladder tumors, hypoxia favours the sialylated O-glycan phenotype (STn antigen) in many cell adhesion proteins including integrins **(Figure 3)** [60]. Moreover, sialylated O-glycans on β4 integrin appear to dynamically regulate different phases of cancer progression: they mediate integrin β4 binding to laminin-332 and β4 phosphorylation, crucial in the metastatic process, while, during Epithelial-mesenchymal transition (EMT), sialylated O-glycans appear down regulated. After EMT, sialylated O-glycans appear to be again upregulated in the mesenchymal state [61].

3. Integrin interaction with glycans present at the ECM-cell membrane microenvironment: implications in cancer

Not only does the glycosylation of the integrin heterodimer affect integrin function but integrins also interact with other glycans present at the ECM-cell membrane interface.

Both N-glycosylation and O-glycosylation of matrix integrin ligands are crucial in mediating the binding to integrins and the activation of signaling cascades in cancer development. The implications of the ECM protein glycosylation in regulating integrin function have been documented in cancer development and embryogenesis [19-26].

Glycosylation of integrin substrates is also crucial in determining the binding and activation of integrins [19-21]. Glycosylation of ECM proteins such as collagens, laminins, and fibronectins, and their interactions with GAGs and other glycans of the glycocalyx (GSLs and other glycoproteins), can modulate integrin activities during cancer progression. **Figure 4** provides a schematic overview of glycan-integrin interactions that can control cancer events.

3.1 N-glycosylation of ECM fibers influences binding with integrins, promoting cell adhesion

N-glycan presence on collagen and laminin influences their binding with the respective integrin receptors **(Figure 4b)**, promoting cancer cell adhesion [8]. In particular, type IV collagen glycosylation effect on integrin binding has recently been investigated: the galactosylation of Hyl393 in α1 (IV) 382- 393 and Hyl540 and Hyl543 in α1 (IV) 531-543 collagen sequences mediate the binding to α3β1 and α2β1 integrins. This results in increased melanoma cell adhesion. Additionally, glycosylation of laminin plays a role in integrin-mediated biological functions. Kariya *et al.,* [9] showed that differential glycosylation of laminin-322 isolated from gastric cancer cells can modulate integrin function. Modification of laminin-332 by bisecting GlcNAc decreases the α3β1 integrin clustering, inhibiting focal contact formation, resulting in decreased cancer cell adhesion and migration. N-glycosylation modifications of human fibronectin has shown *in vitro* its importance in influencing cell adhesion and migration by activating the integrin-mediated signaling [62]. Taken together, these data suggest that N-glycosylation of ECM proteins is also implicated in regulation of integrin-mediated cell fate during cancer and further studies would be crucial in confirming its potential as a therapeutical target.

3.2O-glycosylation of ECM proteins and integrin function: embryogenesis versus cancer

Cancer is often characterised by the activation of molecular pathways and processes normally restricted to embryogenesis [63]. For this reason, studying the glycosylation events in embryogenesis is crucial to identify markers that can be relevant for cancer therapy. O-glycosylation of ECM components, which are integrins ligand integrin ligands?, seem to be essential in embryo development, as well as in cancer progression [64]. The knocking down of polypeptide-N-acetyl-galactosaminyltransferase (ppGalNAcT), that begins O-glycan biosynthesis, caused impaired secretion of integrin ligands which demonstrates the role of O-glycosylation in β1 integrin-mediated signaling during mammalian organogenesis **(Figure 4a)** [65]. O-glycosyltransferase (ppGalNacT-1) deficient mice presented impaired epithelial buds and submandibular glandular growth, reduced secretion of basal lamina components, and a consequent decrease of laminin-mediated signaling through β1 integrin receptors. These events also led to reduced fibroblast growth factor receptor (FGFR1) activation, akt/mapk phosphorylation and reduced epithelial cell proliferation [65].

Furthermore, O-glycosylation of fibronectin seems to have an important role during EMT, a process that occurs in normal development, but it also arises in tumour progression and metastasis [66]. In prostate epithelial cells stimulated with TGF-β, a known inducer of EMT, upregulation of oncofetal fibronectin was observed. OFn is characterised by a certain O-glycosylation at the IIICS domain that is not present in the adult isoform and it is crucial in mediating the signaling underlying EMT. Furthermore, it was shown that β5-integrins are crucial is mediating breast carcinoma cells adhesion during TGFβ-induced EMT [67]. Taken together, these results suggest that aberrant O-glycosylation of matrix proteins have an impact on integrin mediated signaling during the cancer process.

3.3 GAGs and PGs interaction with integrins in cancer

Other ECM glycan based entities regulate integrin activities during cancer: GAGs and Proteoglycans **(Figure 4c)**. The main species involved in this interaction are Hyaluronan (HA) Heparin (HP), Heparan Sulphate (HS) and Condroitin Sulphate (CS)[10, 68-82].

Hyaluronan (HA) is implicated in integrin-mediated mechanotransduction in cancer processes [68, 69]. HA is a major component of the ECM of the brain. Glioblastoma multiforme (GBM), a highly invasive brain tumour, is associated with an increase in HA secretion that leads to tissue stiffening. HA interaction with CD44 receptors contributes to the mechano-transduction in GBM tumour cells, leading to improved adhesion and invasive migration [69].

Heparin (HP) is a GAG that exhibits antimetastatic activity and is already used in the clinic. One of the mechanisms involves the inhibition of cell–cell-interaction through regulating integrins function [70]. HP inhibits melanoma cell metastasis by blocking to α4β1 (Very Late Antigen (VLA-4), which is important for the metastatic dissemination, suggesting VLA-4 as a target [71]. Furthermore, HP can sequester (the) Cyr61 molecule secreted in several malignancies that promote metastasis by activating α4β1-mediated signaling [72].

Heparan Sulfate Proteoglycans (HSPGs) also appear to regulate cancer progression interacting with integrins [10]. In particular, syndecans interact with several integrins mediating various aspects of tumorigenesis. Among syndecans, syndecan -1, -2 and -4 have been studied in several cancer cell lines [73, 75-80].

Syndecan-I promotes growth, migration and tumor angiogenesis, mediating the association of integrins with growth factor receptors (Figure 4c) particularly via interaction of its extracellular region [74, 75].

Syndecan-1 binding with integrins αVβ3 and αVβ5 has been found to induce tumour cell spreading and invasion, in human breast carcinoma cells [76]. In tumor angiogenesis, Syndecan-1 also induces the clustering of integrins αVβ3 and αVβ5 and insulin-like growth factor receptor (IGFR), which leads to intracellular activation of the integrins by the cytoskeletal protein talin, promoting endothelial cell migration [74, 77].

Another consequence of the interaction of syndecan-I with integrins is the mediation of ECM fiber alignment, a cancer phenomenon that directionate the migration and invasion of breast carcinoma cells [78]. Heparan sulphate chains and the ectodomain are necessary to drive the fribronectin fibers alignment mediated by αvβ3 integrins of cancer stromal fibroblast.

If syndecan-1 has a pro-angiogenic effet, syndecan-2 has shown an anti-angiogenic action in tumor angiogenesis. Syndecan-2 shedding occurring on endothelial cells drives changes in β1 integrin activation, resulting in angiogenesis inhibition, and impaired tumor growth [79]. In sharp contrast, this proteoglycan exhibited an opposite effect on breast cancer cells where it induced cell spreading and adhesion, leading to tumour invasiveness. These effects were dependent on Rho GTPases, which regulate the actin cytoskeleton organisation [80].
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 (HER2) that leads to tumor cell survival [75].

Another phenomenon observed in cancer progression that involves proteoglycans is the remodeling of their GAGs chains [81]. Structural modification of GAGs and the interaction with integrins is a key aspect that drives the switch to the tumor-phenotype. Chondrointin Sulphate proteoglycans (CSPGs) have a role in proliferation, migration, and metastasis and they are emerging as relevant therapeutic targets [82]. CSPGs 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 sulphated CS, potenziate (potentiate) sinergistically FGF2 and integrin α5β1 signaling, acting as coreceptor, mediating cell migration.

3.4 Glycocalyx: mechanical and chemical role in regulating integrin functions

The glycocalyx also is involved in cancer cells activities such as adhesion and spreading, influencing integrin interactions and functions [11, 83, 84]. The glycocalyx plays a crucial role in maintaining a cell surface pH nanoenvironmentthat protects cell receptor functions **(Figure 4d)**. The disruption of the glycocalyx by chemical and enzymatic treatments impairs the pH and affects the integrin-mediated migration of melanoma cells [83]. Glycocalyx composition not only affects the chemical environment but also physically influences integrin-mediated cell adhesion [11, 84]. Indeed, the glycocalyx mediates mechanotransduction and the flow-regulated invasion of metastatic cancer cells [84]. In particular, cell surface GAGs, such as HS and HA, together with integrin α3, mediate interstitial flow-induced migration of 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 [11]. This mechanical force guides integrins to the assembly into mature adhesion complexes and plays a role in increasing growth factor signaling associated with metastasis **(Figure 4e)**.

3.5 Carbohydrate-carbohydrates interactions CCIs mediate integrin-glycosphyngolipid complexes

Another aspect of integrin-glycan regulation in cancer is represented by carbohydrate-carbohydrates interactions (CCIs) [85, 86]. 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 CCI interactions between GSLs and glycans carried by integrins. In particular terminal sialylation residues are crucial in CCIs between GSLs and integrin [85]. Highly sialylated ganglioside GT1b has been found to interact with high-mannose residues on α5 subunit of the α5β1 integrin, regulating in this way α5β1-mediated adhesion of epithelial cells to fibronectin [86]. GT1b also interacts with glycans present on integrin α2, and modified sialylation is associated with tumour progression. α2,3-sialylation of α2 subunits were required for the integrin α2β1-dependent cell adhesion to Cn type I, and the same α2,3-linked sialic acid residues on the integrin receptor were responsible for the interaction with the carbohydrate moiety of AsGM1, accounting for the complex formation between AsGM1 and α2β1 integrin receptors[50]. This evidence provides novel insights into the role of sialic acids in the organization and function of important membrane components in invasion and metastatic processes**.**

4. Concluding remarks: importance of further understating the integrin relationship with the cancer glycomicroenvironment

A wide range of data demonstrates the crucial role of glycans in regulating integrin function, in both physiological and pathological conditions. This regulation is explained by both the presence of glycans on integrin subunits, and by other glycan components present at the extracellular surface. Among the 24 integrins, only types α5β1, α2β1, α3β1, αvβ1, α4β1, α4β6, α6β1 have been characterised for the glycans present on their structure.

N-glycan species were most expressed on these integrin structures, and they were important in mediating integrin transport on cell membrane, activation, dimer-formation, and in binding with the substrate. The remodelling of these sugar moieties carried out by the glycosyltransferases is implicated in the regulation of cell motility and in the migration associated with cancer formation and progression.

This phenomenon is considered as one of the cancer mechanisms since it has been observed in several cancer cell lines, and altered general glycosylation has been observed in cancer patients. Also, O-glycans present on integrin structures have shown the capacity to regulate the neoplastic mechanism; however, there are fewer studies of this type of glycosylation because of the limitation in the experimental techniques for O-glycan characterization.

The interaction of integrins with other glycan species present at the ECM-cell interface regulates integrin activities involving several mechanism; some studies have shown that the glycosylation of integrin ligands and their counterparts are also crucial in cell tumor processes. Interestingly, the whole glycocalyx composition can also modulate integrin activities influencing the Ph and the mechanical forces applied and this has also been correlated with cancer invasiveness.

New analytical tools and methods are needed to elucidate not just the structural changes of glycosylated integrins, but also the causal link between integrin glycosylation and cell fate control. The development of new platforms and new models in which glycosylation of integrins and interactor partners can be studied is necessary to clarify the cancer progression mechanism. Future studies with such affordable models better able to mimic the glycomicroenvironment, could be useful in the investigation of glycosylation impact on integrin mediated signaling in physiological and tumor states.

Acknowledgements

The authors acknowledge the facilities and scientific and technical assistance of the Centre for Microscopy & Imaging at the National University of Ireland Galway (www.imaging.nuigalway.ie), a facility that is funded by NUIG and the Irish Government's Programme for Research in Third Level Institutions, Cycles 4 and 5, National Development Plan 2007-2013.This material is based upon works supported by the European Union funding under the 7th Framework Programme under Grant Agreement Number 317304.This publication has emanated from research supported in part by a research grant from Science Foundation Ireland (SFI) and is co-funded under the European Regional Development Fund under Grant Number 13/RC/2073. We also acknowledge the editorial assistance of Mr Keith Feerick and Mr Anthony Sloan, and the graphic support of Mr Maciej Doczyk.

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Trends

- Integrins have a well recognised role in cancer, controlling cell stemness, adhesion, migration, survival, and promoting metastasis.
- Glycans are strongly involved in integrin stability and functionalities.
- Glycosylation of integrins and their interaction partners is misregulates in tumorigenic processes.

Glossary

Integrins: a large family of heterodimeric receptors comprising 24 αβ that mediate cell attachment to the extracellular matrix (ECM) but also take part in specific cell-cell interactions.

Glycosylation: complex post-translational modifications (PTMs) that allow the attachment of glycans to proteins, lipids and other saccharides, regulating their function.

N-glycans: glycans linked to asparagines residues on proteins through an N-acetylglucosamine (GlcNac).

O-glycans: attached to threonine, serine or tyrosine on proteins through N-acetylgalactosamine (GalNac) and in some cases through mannose (Man) and fucose (Fuc).

Glycosaminoglycans (GAGs): linear structures, sulphated, negatively charged polysaccharides composed of disaccharide repeating units: a uronic acid (D-glucoronic acid (D-GlcA) or L-iduronic acid (IdoA)) and an amino sugar (D-galactosamine (D-GalN) or D-glucosamine(D-GlcN)). GAGs are present and involved in the functional regulation of the ECM itself.

Hyaluronic acid (HA): a GAG defined by the disaccharide unit (GlcNAcβ1–4GlcAβ 1–3) $_n$ that is neither sulfated nor covalently linked to protein.

Chondroitin sulphate (CS): a GAG defined by the disaccharide unit (GlcNAcβ1–4GlcAβ 1–3) $_n$ that is neither sulfated nor covalently linked to protein.

Heparin (HP): A type of heparan sulfate made by mast cells that has the highest amount of iduronic acid and of N- and O-sulfate residues.

Heparan sulphate (HS): A GAG defined by the disaccharide unit (GlcNAcα1–4GlcAβ1–4/IdoAα1–4) $_n$ containing N- and O-sulfate esters at various positions, and typically found covalently linked to a proteoglycan core protein.

PGs: a class of glycoproteins carrying GAGs linked through a covalent bond to threonine/serine.

Glycosphyngolipids (GSLs): molecules composed of a core of β-linked glucose or galactose associated with the ceramide.

Glycosylphosphatidylinositol (GPI) anchor: a complex PTM of proteins in the outer layer of the membrane consisting of a phospholipid molecule, a glycan core and a phosphoethanolamine (Etn) linker.

Glycocalyx: on the surface of all eukaryotic cells, it is composed of PGs, glycoproteins and glycosphyngolipids (GSLs) associated with the plasma membrane, as well as soluble GAGs.

Outstanding questions

- Are data obtained from integrin glycosylation are enough to characterize glycan impact in cancer? Is N-glycosylation the major contributor to the process?
- Is cell fate mis-regulation in tumor progression due to glycan content and structures on integrin subunits? Is it possible to modulate these changes and thereby restore the physiological glyco-microenvironment?
- What are the best and most affordable models by which to study integrin glycosylation impact in cell-cell and cell-ECM events regulated by glycans?
- How can the role of integrin glycosylation and glyco-microenvironment be used in the design of new diagnostic and therapeutic strategies?

739 **List of Figures**

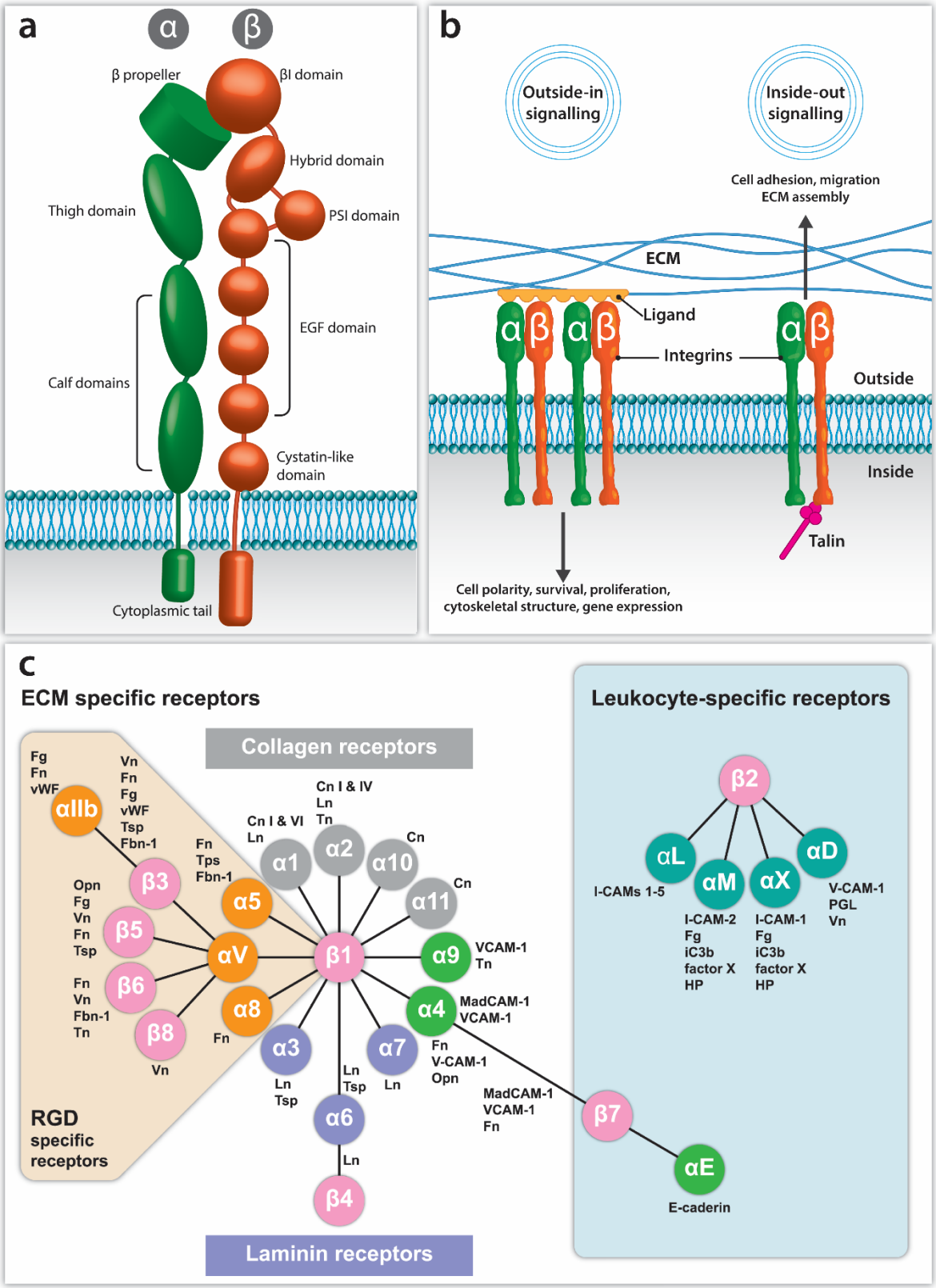

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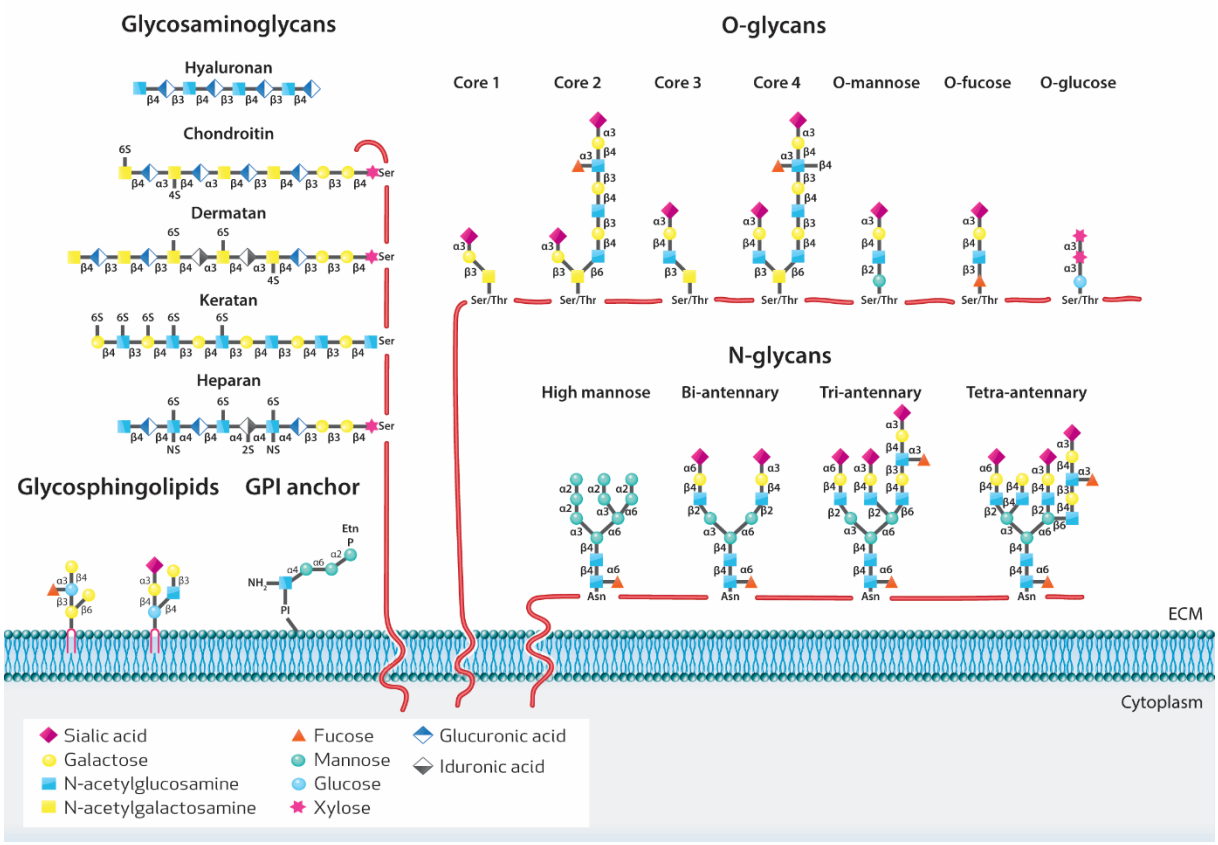
Figure 1: Overview of integrin structure, signaling and specificity: (a) Integrins are membrane receptors consisting of an α- and a β-subunit. Each subunit has a large extracellular domain which binds ligands, a single transmembrane helix and a short cytoplasmic portion. Both α and β subunits consist of different sub-domains. 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 signaling:** Integrins bind ligands in the extracellular space, triggering an 'outside–in' signaling that controls cell polarity, cytoskeletal structure, gene expression, cell survival and proliferation. Also, they can mediate an 'inside-out signaling triggered by an intracellular activator such as talin that binds to the β-integrin tail. This results in increased affinity for ECM, cell migration and ECM remodeling and assembly. **(c) Schematic representation of integrin specificity:** In mammals, 18α- and 8β- integrin isoforms combine into 24 αβ receptors. They can be grouped based on their preferred ligand or they can be grouped into ECM binding receptors and leukocyte binding receptors. The ECM binding receptors can be sub-categorized into collagen, fibronectin and RGD-binding receptors. Every single receptor also has the capability to bind other cellular or non cellular molecules. Cn denotes collagen; HP denotes heparin; Fbn-1 denotes fibrillin 1; Fn denotes fibronectin; Fg denotes fibrinogen; Ln denotes laminin; vWF denotes von Willebrand factor; Opn denotes osteopontin; Tn denotes tenascin; Tsp denotes thrombospondin; Vn denotes vitronectin; VCAM-1 denotes Vascular Cell Adhesion Molecule 1; ICAM-I denotes Intercellular Adhesion Molecule 1; MadCAM-1 denotes mucosal vascular address in cell adhesion molecule 1. iC3b denotes a proteolytically inactive product of the complement fragment C3b.



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775 **Figure 2: Mammalian glycans species present at the cell membrane-ECM:** The main
776 classes of glycans, glycosaminoglycans, N-glycans, O-glycans, glycophyngolipids,
777 glycosylphosphatidylinositol (GPI) anchor. GAGs, heparin sulphate (HS) chondroitin sulphate
778 (CS) hyaluronic acid (HA), dermatan sulphate (DS), keratin sulphate (KS), are depicted. NS,
779 2S, 4S and 6S represent the sulphation positions on the GAGs chains. Representative examples
780 of complex-type N (Bi-tri-tetra-antennary) and high-mannose N-glycans and are illustrated.
781 Core 1–4 O-glycans are depicted, as well as O-mannose, O-fucose and O-glucose structures.
782 Glycan linkages are identified by the anomeric configuration (α or β) of the donor saccharide
783 and by the ring position (1–6) of the acceptor sugar. The GPI anchor and examples of
784 glycosphyngolipids are also represented. Etn–P denotes a phosphoethanolamine and PI is
785 phosphatidil inositol. Asn denotes asparagine; Ser denotes serine and Thr denotes threonine.

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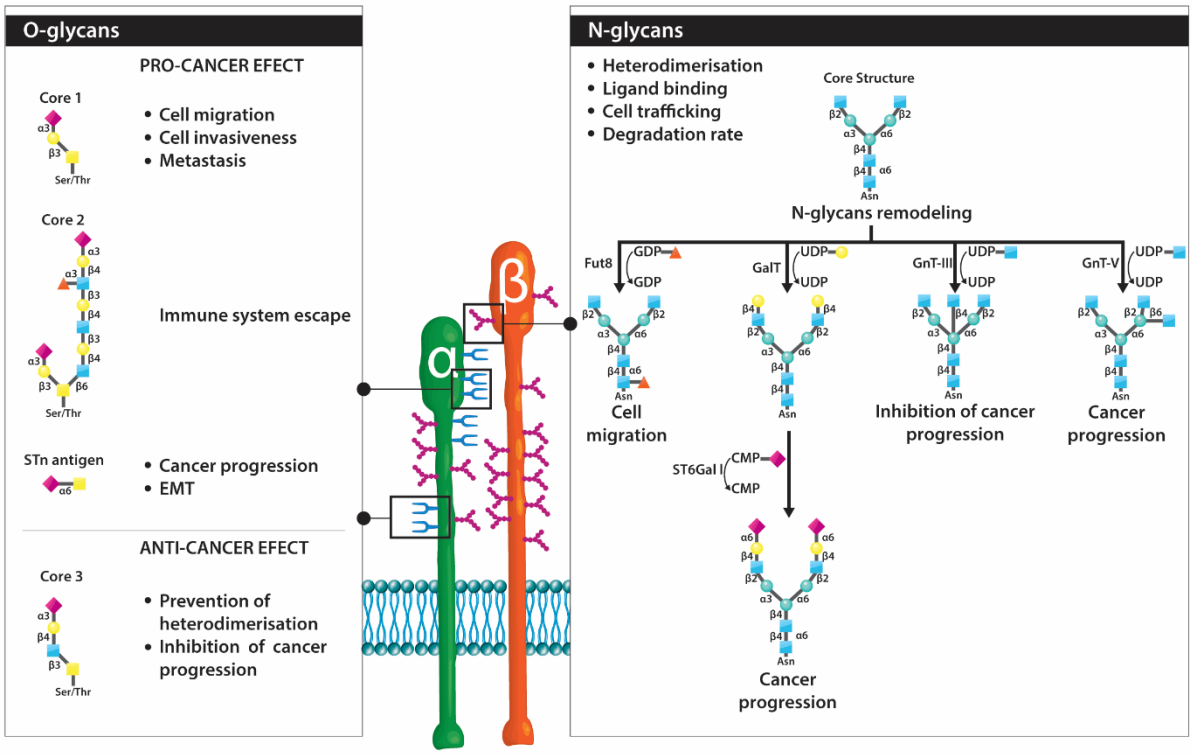

Figure 3: Integrin glycosylation and glycan moieties remodeling, through glycotransferases actions, and the effect on integrin function: Core 1, core 2 O-glycosylation and STn antigen on integrins are associated with cancer progression while core-3 glycosylation prevents the dimerisation of α and β chains. N-glycan core structure is involved in heterodimerisation, ligand binding, cell trafficking and the degradation rate of integrins. Glycan remodeling occurs through glycosylation reactions by glycosyltransferase. GnT-III denotes β 1,4-N-Acetylglucosaminyltransferase III; GnT-V denotes β1,6 N-acetylglucosaminyltransferase V; GalT denotes Hydroxyproline-O-galactosyltransferase; ST6GalI denotes ST6 β -galactoside alpha-2,6-sialyltransferase 1 and Fut 8 denotes α1,6-fucosyltransferase. Remodeled 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. In contrast, overexpression of GnT-III down-regulates integrin-mediated cell migration. ST6GalI overexpression is associated with cancer progression while Fut8 has a role in cell migration.

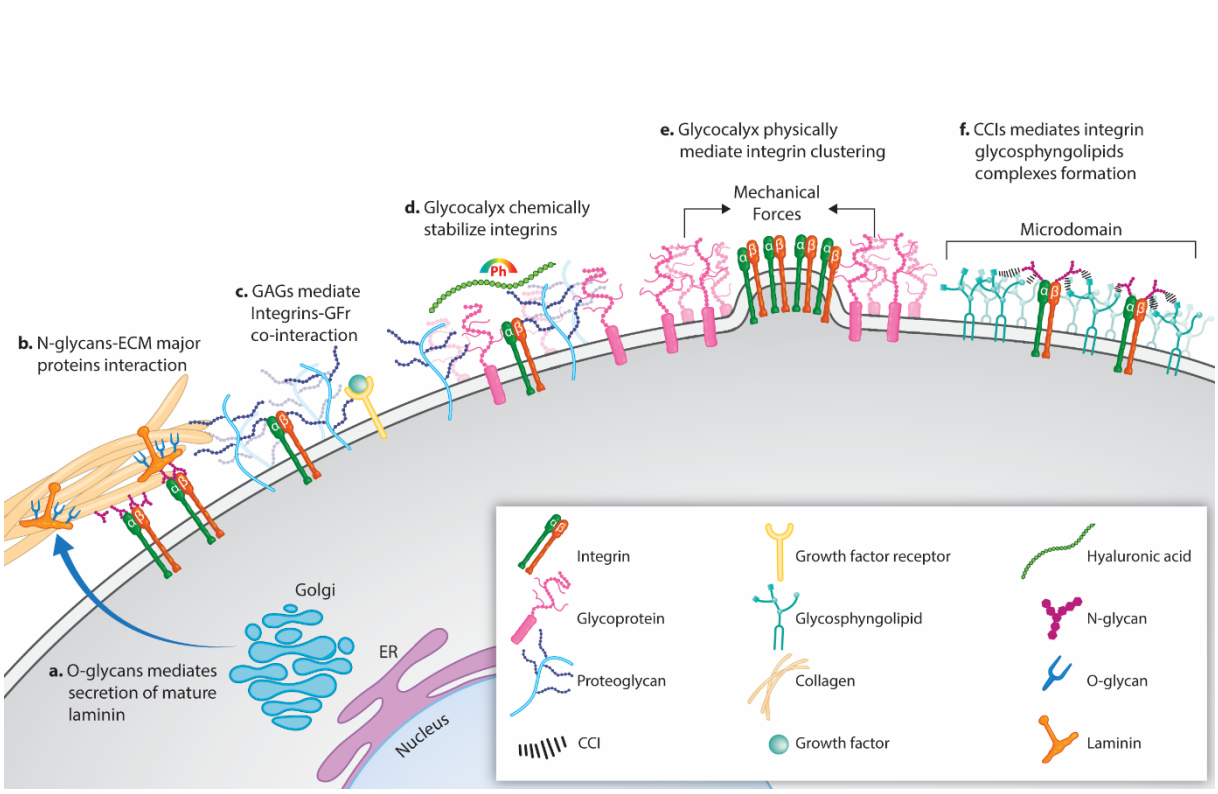

Figure 4: Integrin-glycan interactions at the ECM-cell microenvironment occurring in cancer. (a) O-glycosylayion is crucial in the secretion of mature laminin, integrin ligand. **(b)** N-glycosylation of ECM major proteins such as laminin and collagen mediates the binding with integrins receptors**. (c)** The interaction between GAGs and integrins activates integrins (themselves) and mediates the coupling with growth factor receptors. **(d)** The glycocalyx preserves integrin conformation maintaining the pH. **(e)** Mechanical action of the glycocalyx in mediating integrin clustering. **(f)** Carbohydrate interactions between the glycans present on integrins and the glycosphyngolipids mediate the formation of microdomains that are crucial in signal transduction.

Integrin	Glycosylation	Function associated to glycosylation	System	Carrying-glycans domain	References
α5β1	N- glycans core structure	Association of α and β subunits	K562 leukemia cells		[20]
		Transport on cell membrane Presence of β1 subunits on the membrane	H7721hepatocarcinoma cells	β1	[23]
		Active α5β1 expression, and internalization	Breast cancer cells	β-propeller	[18]
		Cell spreading and migration Laminin interaction	HeLa cervical cancer cells	Calf1-2	[19, 22]
		EGFR complex formation			
	Bisecting GlcNac	Decreased binding affinity to fibronectin Inhibition of cell spreading and migration	B16-hm highly mestatatic melanoma cells		[26]
		Suppression of metastasis			
	Branched β1,6 GlcNAc	Metastasis potential	MT1, MTAg, and MTPy leukemia cells		[27]
		Migration on fibronectin	Uveal WM1205Lu melanoma cells		[36]
		Suppression of metastasis	GnT-V-/- mice		[28]
	Terminal α2,6 hypersialylation	Collagen-I and fibronectinn binding	HD3 colonocyte	β1	[49]
		Cell motility	SW48 colon epithelial cells		[40]
		Cancer progression	Human colon adenocarcinoma	β1	
	α2,8 sialylation	Cell adhesion to fibronectin	Human melanoma cell line G361	α5	[46]
	Poly-N-acetyllactosamine	Suppress the activation of β1 integrin	HCT116, SW480, SW620, Colo205 and HT29	β1	[87]
		β1 increased expression delayed degradation	Neuroblastoma cells	β1	[88]
		FK phosphorylation Migration, invasion, tumor growth			
α2β1	α2,3 sialylation	Interaction and adhesion to Cn-I and AsGM1	C4-2B prostate cancer cells	α2	[50]
		Metastasis formation			
	O-Glycan Core 1	Enhanced invasiveness	Hepatocarcinoma cells SK-Hepl HepG2 HA22T HCC36	β1	[54]
	O-Glycan Core 3	Prevented heterodimerisation	Prostate carcinoma PC3	β1	[58, 59]
		Inhibition of tumor formation and metastasis Inhibition	Gastrointestinal LNCaP		
α3β1	Bisecting GlcNac	Reduced migration	MKN45 cells		[35]
	Branched β1,6 GlcNAc	Enhanced cell adhesion	WM1205Lu		[36]
		Increased metastatic potential	Melanoma cells		
		Migration on Fibronectin Association with CD151 tetraspanin	B16BL6 cells		[33]
		Cell spreading and motility			
	Core fucosylation	Cell migration and signaling	Liver cancer HepG2 cell	β1	[52]
	α2,6-linked sialic acid				
	Tri- and tetra-antennary β1,6-Galβ1- 4Glc	Binding to vitronectin	Melanoma cells		[47]

Integrin	Glycosylation	Function associated to glycosylation	System	Carrying-glycans domain	References
αvβ3	Tri- and tetra-antennary β1,6-Galβ1- 4Glc α2,6 and α2,3 Sialic acid	Migratory ability Binding to vitronectin	WM9 cells		[47]
	High level bisecting GlcNAc High-mannose α2,6-linked sialic acid	Migratory capacity	WM239 cells		[47]
	Lower level bisecting GlcNAc High-mannose α2,3-linked sialic acid	Migratory capacity	WM793 cells WM1205Lu cells		[48]
α4β1	Terminal sialylation	Fibronectin binding	Human Burkitt's lymphoma HBL-8 cells	β1	[43]
	β-galactose	ECM invasion Increased motility Metastasis			[43]
α6β1	β 1,6-branched oligosaccharides	Impared integrin presence on the membrane	NIH 3T3 fibroblasts	α6	[31]
		Impared binding	Murine melanoma B16-F10 cells		[29, 30]
α6β4	Branched β 1,6 GlcNAc	Modulation of adhesion and motility	MKN45 cells	β4	[34]
	O- sialylation	Binding to laminin β4 phosphorylation, metastasis, ETM	Human keratinocyte HaCaT cells		[61]

Table1: Integrin glycosylation: This table summarises the glycan species present on integrin structure, and the functional role associated with it.