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REVIEW

Don't sugarcoat it: How glycocalyx composition influences cancer progression

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Mechanical interactions between tumors and the extracellular matrix (ECM) of the surrounding tissues have profound effects on a wide variety of cellular functions. An underappreciated mediator of tumor–ECM interactions is the glycocalyx, the sugar-decorated proteins and lipids that act as a buffer between the tumor and the ECM, which in turn mediates all cell-tissue mechanics. Importantly, tumors have an increase in the density of the glycocalyx, which in turn increases the tension of the cell membrane, alters tissue mechanics, and drives a more cancerous phenotype. In this review, we describe the basic components of the glycocalyx and the glycan moieties implicated in cancer. Next, we examine the important role the glycocalyx plays in driving tension-mediated cancer cell signaling through a self-enforcing feedback loop that expands the glycocalyx and furthers cancer progression. Finally, we discuss current tools used to edit the composition of the glycocalyx and the future challenges in leveraging these tools into a novel tractable approach to treat cancer.

Introduction

The mechanical interactions between a cell and the ECM tissue that encompasses it control nearly all aspects of cellular fate (Daley et al., 2008). The glycocalyx, the thick mixture of protein, lipids, and their post-translational sugar structures, surrounds all living cells and acts as a buffer between the cell and the ECM, especially in terms of mechanics (Butler and Bhatnagar, 2019). In cancer, the size of the tumor cell glycocalyx as a whole is significantly increased (Pavelka and Roth, 2010), and this in turn alters all aspects of tumor progression including transmembrane receptor function, cellular tension, integrin-mediated signaling, cell–cell and cell–ECM interactions, and immune recognition (Uchimido et al., 2019). On the other hand, the composition of the glycan structures decorating the protein and lipid backbones during cancer is context dependent, as the glycan trees are either elongated or truncated based on the specific cancer (Munkley and Elliott, 2016). Regardless, the composition of these sugar structures in the glycocalyx plays an important role in regulating both the overall phenotype and mechanics of the tumor (Martinez-Seara Monne et al., 2013). This review will discuss both the protein and lipid backbones that comprise the glycocalyx and also the critical glycan structures attached to these backbones, which are altered during cancer progression. Furthermore, we will detail how mechanics modulates the structure and function of the cancer glycocalyx and how this drives a "feedback loop" which drives malignancy. Finally, we will discuss current strategies to "prune" the glycocalyx in a specific manner to modulate cancer progression.

Key protein and lipid backbones of the glycocalyx in vivo

The composition and structure of the glycocalyx, a heterogeneous mixture of proteins and lipids that extend away from the cell membrane to which they are anchored, affect nearly all interactions between the cell and the extracellular environment. The height of the glycocalyx varies widely between cells and tissues but in general ranges from tens of nanometers to several micrometers thick (Möckl et al., 2019). The proteins and lipids of the glycocalyx have bulky post-translational sugar structures decorating their surface that extend the height and bulkiness of the glycocalyx and give it a strong negative charge (Reitsma et al., 2007). Cell surface chemokine receptors and integrins that are encompassed by the glycocalyx are much shorter (~10 nm; Ye et al., 2010) and must navigate this negative charge and the repulsion between the ECM and glycocalyx, in order for cellular adhesion, migration, signaling, and most any cell-surface interactions to occur (Hammer and Tirrell, 1996).

The protein and lipid backbones of the cellular glycocalyx comprise four main classes with unique glycosylation patterns (Fig. 1): mucins, which are glycoproteins with bulky

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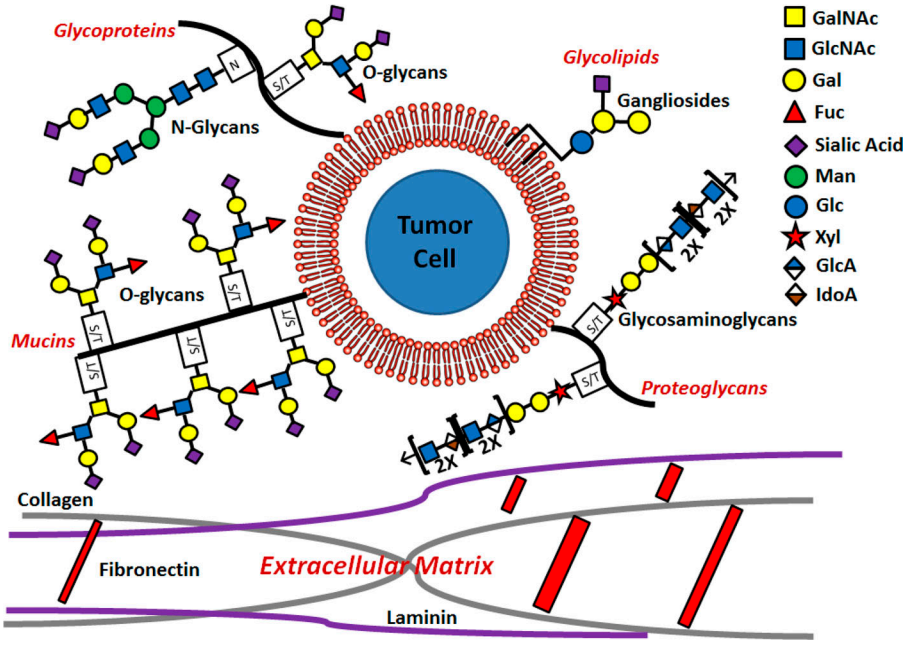

Figure 1. **Structure of the tumor cell glycocalyx.** The glycocalyx is the first line of contact between the tumor cell and the components of the ECM such as fibronectin, collagens, and laminin. The cancer cell glycocalyx consists of four main glycan branches on four distinct types of protein or lipid backbone: O-glycans attached to glycoproteins and mucins at serine/threonine sites, N-glycans attached to glycoproteins at asparagine sites, gangliosides attached to ceramide glycolipids, and GAGs characterized by the Xyl-Glc-Glc motif attached to a protein at a serine/threonine site on proteoglycans.

O-linked glycan attachments that influence integrin function and cell signaling; trafficking glycoproteins, which primarily regulate cell adhesion through N- and O-linked structures; glycolipids, which consist of ganglioside attachments to ceramides; and proteoglycans, which are characterized by glycosaminoglycans (GAG) attachments. Each of these classes is discussed separately below.

Mucins

The mucins are critical glycoprotein components of the glycocalyx that form a gel-like mucus on the surface of cells that modulates a variety of cellular interactions including integrin clustering, tension sensing, and signaling (Kufe, 2009). Mucins consist of rather lightly glycosylated N- and C-terminal domains flanking a central region containing a massive amount of O-glycosylation, which increases the overall molecular weight of the glycoprotein and makes mucins uniquely resistant to degradation (Bansil et al., 1995). The sheer amount of glycosylation on mucins makes them especially susceptible to changes during cancer progression as there are countless sites for aberrant glycans. In particular, the mucins MUC1 and MUC16 have been implicated in driving cancer progression. MUC1 is a key oncomarker and is critical for maintaining the cancer stem cell population (Nath and Mukherjee, 2014). During breast and other cancers, MUC1 becomes even more heavily glycosylated than in normal tissues, with many more O-glycan sites occupied, and adds to the overall bulk of the glycocalyx (Müller et al., 1997; Taylor-Papadimitriou et al., 1999). Furthermore, MUC16 is critical for shielding cancer cells from natural killer (NK) cells during innate immune surveillance (Gubbels et al., 2010). Recent work has described how synthetic glycopolymers that truncate either MUC1 or MUC16 serve to de-bulk the glycocalyx and slow cancer progression in response to mechanical stimuli (Paszek et al., 2014; Woods et al., 2017).

PSGL-1, CD43, and CD44, the glycoprotein regulators of cell adhesion

The glycoproteins P-selectin glycoprotein ligand-1 (PSGL-1), CD43 (leukosialin), and CD44 are components of the glycocalyx that act as regulators of cell–cell, cell–endothelial, and cell–ECM interactions in cancer (Spertini et al., 2019). PSGL-1 is a cell surface glycoprotein found on all leukocytes and some cancers that is a critical regulator of selectin binding to the endothelial surface (McEver and Cummings, 1997). It binds to E-, P-, and L-selectin at varying affinities and can carry both sialyl Lewis x (sLe^x) and sialyl Lewis a (sLe^a) glycans to confer this binding (Moore, 1998). PSGL-1 has been shown to have profound effects on immune recognition and cancer progression as its deletion has been shown to up-regulate macrophage cytokine production and colorectal cancer formation (Li et al., 2017), prevent T cell exhaustion and improve outcomes in melanoma (Tinoco et al., 2016), and attenuate trafficking to the lung and platelet adhesion in epithelial cancers (Kim et al., 1998).

CD43 or leukosialin is a glycoprotein constituent of the glycocalyx that acts as a selectin-dependent trafficking receptor (Matsumoto et al., 2007) and is expressed on all hematopoietic subsets except some B cell populations (Carlsson and Fukuda, 1986; Fukuda and Carlsson, 1986). Aberrant glycosylation of CD43, detected by the biomarker UN1, is a hallmark of many lymphoid cancers (Tuccillo et al., 2014) and also solid tumors in the breast, colon, gastrointestinal tract, and lung (Tassone et al., 2002). Furthermore, overexpression of CD43 has been implicated in an increase in the p53 tumor suppressor and enhanced p53-mediated cell death of colon cancer (Kadaja et al., 2004).

Finally, CD44 is a glycoprotein expressed on pancreatic and breast tumors (Li et al., 2014) that binds its principal ligand, hyaluronan (hyaluronic acid), but also collagen and fibronectin in the ECM (Chen et al., 2018). CD44-HA binding of tumors leads to an up-regulation in cell proliferation and cell motility (Ponta et al., 2003) in the tumor through activation of the MAPK and phosphoinositide 3-kinase pathways (Lv et al., 2016). Changes in

the association of CD44 with the actin cytoskeleton through the Ezrin-Radixin-Moesin protein Ezrin have been implicated in both breast (Donatello et al., 2012) and pancreatic (Meng et al., 2010) cancer progression through both loss of cell–cell contacts and altered growth factor signaling (Clucas and Valderrama, 2014). Furthermore, CD44 has up to 10 splice variants termed CD44v1–10 that are alternatively expressed in other cancers, such as head and neck (Reategui et al., 2006), prostate (Ni et al., 2014), colorectal (Todaro et al., 2014), bladder (Kobayashi et al., 2016), and gastric (Lau et al., 2014), and are correlated with increased cancer progression and poorer patient outcomes (Mulder et al., 1994). Finally, CD44 is able to switch between the CD44s and CD44v forms (Brown et al., 2011), and epithelial to mesenchymal transition (EMT) in pancreatic and breast tumors requires a switch from CD44v to CD44s (Zhao et al., 2016).

Glycolipids

In addition to the glycan structures attached to proteins that we have discussed, glycans attached to lipids play a critical role in cancer progression and are susceptible to manipulation during cancer progression (Daniotti et al., 2013). There are two main classes of glycolipids: glyceroglycolipids, which contain a glycerol and a fatty acid as the lipid, and glycosphingolipids (GSLs), which carry a sphingosine as the lipid (Lopez and Schnaar, 2006). Since glyceroglycolipids are only found in plants, we will focus on GSLs, which are divided into several subclasses, including cerebrosides, which are singly glycosylated GSLs (Glc-Cer or Gal-Cer), globosides, which are poly-glycosylated GSLs (Lac-Cer), and gangliosides, which contain at least one sialic acid attached to the Lac-Cer structure (Schnaar, 2019; Schnaar and Kinoshita, 2015). GSLs play a critical role in tumor progression in the brain, an organ with complex and abundant gangliosides (Hirabayashi, 2012), but also in the bone, skin, and lung (Daniotti et al., 2016). GSLs also play roles in escaping tumor immunosurveilance by repressing monocyte cytokine release (Heitger and Ladisch, 1996), preventing release of IgG and IgM from B cells (Kimata and Yoshida, 1994), and limiting $CD8^+$ T cell production (McKallip et al., 1999). Furthermore, GSLs increase tumor cell trafficking as they carry sLe^x structures, which confer E-selectin binding, the master regulator of leukocyte and tumor cell trafficking, through the endothelium (Mondal et al., 2016; Nimrichter et al., 2008).

Proteoglycans

Proteoglycans are the last major component of the glycocalyx and are characterized by a protein backbone with abundant O-linked GAG attachments (Nikitovic et al., 2018). The most abundant GAG attachments include heparin sulfate, chondroitin sulfate, or keratin sulfate and are characterized by the galactose-galactose-xylose motif (Gal-Gal-Xyl; Pomin and Mulloy, 2018). Several tumor-associated proteoglycans have been implicated in cancer signaling and progression, including the Syndecan (Cheng et al., 2016a) and Glypican (Li et al., 2018) families, and their roles are discussed in great detail in the cited reviews.

Critical glycan moieties aberrantly expressed in cancer

In terms of the glycocalyx content, it is not only the protein and lipid backbones that are important but also the post-translational sugar structures attached to the backbones (Pinho and Reis, 2015). After the proteins and lipids are synthesized ER, they move to the Golgi, where they are post-translationally modified by the combination of activated sugar donors and enzymes called glycosyltransferases before being transported to the cell surface (Stanley, 2011). This leads to the diverse set of glycan structures which, when presented on the surface of the protein and lipid backbones, form the typical "brush"-like structure of the glycocalyx (Kabedev and Lobaskin, 2018). There are several main glycosylation types on the backbones of the glycocalyx. Glycoprotein glycosylation comprises two main types: the branched N-glycan chains attached to asparagine and the step-wise constructed O-glycan chains attached to either serine or threonine. Finally, glycolipids have glycans attached to a lipid and are initiated by a glucosamine attached to the ceramide backbone (Glc-cer; Varki, 2017). The composition of these glycan structures is not only critical in regulating tumor cell function as a whole but also modified in tumors as compared with normal cells. To this end, specific aberrant glycan structures have been implicated as playing a regulatory role in cancer progression (Fig. 2), and they are outlined in the next section.

Terminal sialylation with ST3Gal-1, ST6Gal-1, and ST6GalNAcs

Sialylation is a critical modification to glycan chains as the addition of a sialic acid both terminates further glycosylation on the chain and is the only sugar that carries a (negative) charge (Wopereis et al., 2006). Altered sialylation is a hallmark of cancer progression, and which class of sialyltransferase adds the sialic acid plays a key role to this progression. To this end, the sialyltransferases ST3Gal-1, ST6Gal-1, and the ST6GalNAcs all add sialic acid to specific glycan structures and have been implicated in cancer. ST3Gal-1 is a sialyltransferase that catalyzes the terminal addition of a sialic acid in a 2,3 linkage to the galactose and terminates the O-glycan. High levels of these short core 1 O-glycan structures have been implicated in a variety of cancers including breast (Yeo et al., 2019), ovarian (Wu et al., 2018), prostate (Tzeng et al., 2018), and brain (Chong et al., 2015). ST3Gal 1 is thought to be up-regulated during tumorigenesis (Picco et al., 2010), and the truncated glycan created interacts with galectin-4, which promotes metastatic signaling (Tzeng et al., 2018).

ST6Gal-1, which adds a sialic acid in a 2,6 linkage on N-linked glycans, causes drastically different functions in cancer, especially in terms of resistance to treatment and survival (Garnham et al., 2019). Sialylation by ST6Gal-1 is thought to maintain a more stem-like state in cancer cells (Schultz et al., 2016) and has been implicated in bladder (Antony et al., 2014), colon (Zhang et al., 2017), breast (Lu et al., 2014), and ovarian cancers (Christie et al., 2008). Multiple works have demonstrated how ST6Gal-1 both confers resistance to chemotherapy (Britain et al., 2018; Schultz et al., 2013) and protects cancer cells from apoptosis by sialylation of Fas (Swindall and Bellis, 2011) and tumor necrosis factor receptor (Holdbrooks et al., 2018). Interestingly, ST6Gal-1 is also the only glycosyltransferase to date in which its soluble form can "extrinsically" glycosylate outside the cell (Manhardt et al., 2017). This mechanism describes how donor sugar from

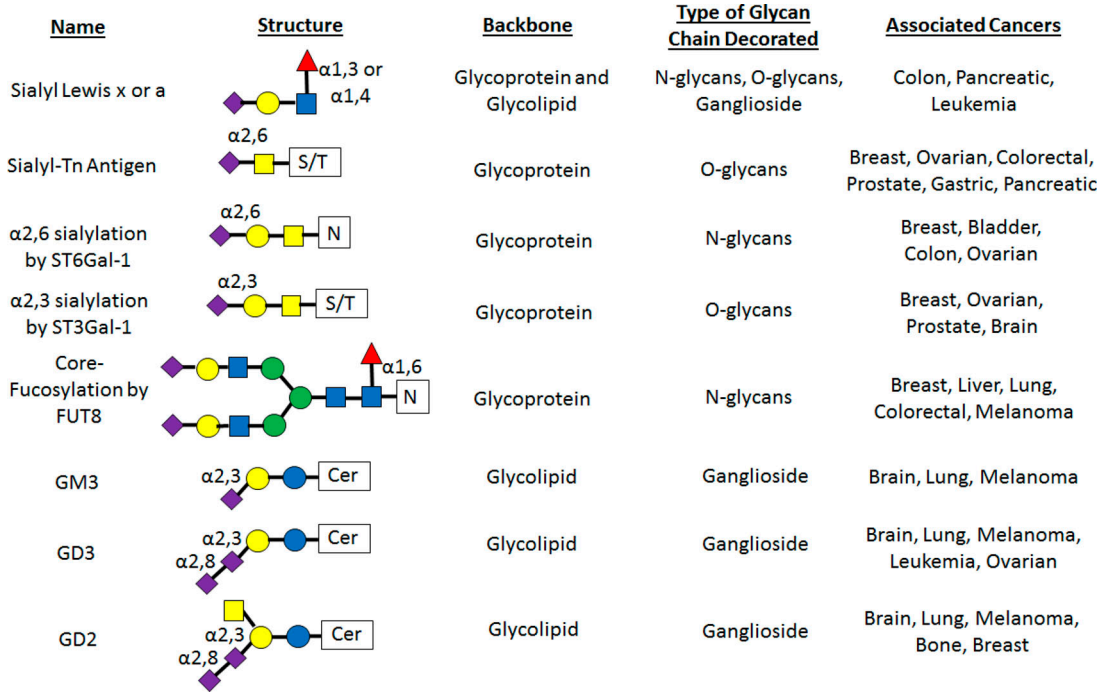

Figure 2. **Principal glycan structures associated with cancer.** A listing of the most common glycosylation structures seen during cancer including sLe^x and sLe^a, sialyl-Tn antigen, α2,6 sialylation by ST6Gal1, α2,3 sialylation by ST3Gal1, core-fucosylation by FUT8, and the GM3, GD3, and GD2 structures. The name, structure, which glycocalyx backbone it decorates, class of glycan, and which cancers it is associated with are listed for each glycan.

platelets (Lee-Sundlov et al., 2016; Lee et al., 2014) and ST6Gal-1 from B cells (Irons and Lau, 2018; Irons et al., 2019) can sialylate progenitors in the bone marrow (Nasirikenari et al., 2014) and prevent granulopoiesis (Dougher et al., 2017). In fact, this "extrinsic" sialylation mechanism has recently been implicated in colon cancer progression, as ST6Gal-1 loaded into exomeres can both sialylate β1 integrins and promote cancer organoid growth (Zhang et al., 2019).

Finally the ST6GalNAc family of sialyltransferases catalyzes the addition of a 2,6 sialic acid to the initial GalNAc instead of the galactose-like ST3Gal-1 and ST6Gal-1 and creates the sialyl-Tn antigen that is expressed in a wide variety of cancers (Marcos et al., 2004). ST6GalNAcs are thought to be regulators of malignancy as they form the binding epitopes for galectin-3 and other galectins as well (Dimitroff, 2015, 2019) and compete away possible core 2 O-glycan extensions such as sLe^x (Lo et al., 2013b). In sum, the interplay between these three structures in preemptively terminating O- and N-glycosylation affects almost all aspects of cancer progression.

Sialyl Lewis antigens

The sialyl Lewis antigens, especially sLe^x and sLe^a, form the binding epitopes to the endothelial selectins on immune cells (Buffone et al., 2013; Mondal et al., 2015) and circulating tumor cells (Burdick et al., 2012; Li and King, 2012). These tetrasaccharide structures, consisting of sialic acid bound to a lactosamine (Gal-GlcNAc) in a 2,3 linkage and a fucose bound in either a 1,3 or 1,4 linkage (Trinchera et al., 2017), are highly implicated in the metastasis of tumors to secondary sites through the bloodstream (Blanas et al., 2018; Mondal et al., 2018). They are strongly up-regulated in a variety of tumors, and sLe^a (CA19.9) and sLe^x (NCC-ST-439) are prognostic markers for both colon and pancreatic cancer (Shiozaki et al., 2011). Furthermore, enforced fucosylation of hematopoietic stem and progenitor cells (or other immune cells; Buffone et al., 2017; Videira et al., 2018) generates sLe^X structures on CD44 to create the hematopoietic cell E- and L-selectin ligand epitope, which is the principal regulator of trafficking to the bone marrow compartment (Dimitroff et al., 2001a,b; Sackstein, 2012; Zhao et al., 2016).

Core fucosylation of N-glycans

Another critical regulator of cancer invasiveness and progression is the core fucosylation of N-glycans. The addition of a 1,6 linked fucose to the chain initiating GlcNAc on complex N-glycans is catalyzed by the fucosyltransferase, FUT8 (Yang et al., 2017). FUT8-mediated core-fucoylation has been implicated as a driver of cancer invasiveness in breast cancer by fucosylation of TGF-β (Tu et al., 2017), in colorectal cancer through a p53-mediated mechanism (Noda et al., 2018), in melanoma by fucosylation of L1CAM (Agrawal et al., 2017), through a β-catenin– and LEF-1–dependent mechanism in nonsmall cell lung cancer (Chen et al., 2013), and through miRNA regulation of fucosylation in hepatocellular carcinoma (Cheng et al., 2016b).

GM3, GD2, and GD3 gangliosides

Mammalian GSL glycosylation is characterized by the initial addition of a glucosamine (Glc-Cer) to the ceramide followed by a galactose to form Lac-Cer (Merrill, 2011). It is from this basic glycan structure that all of the gangliosides, or sialic acid–containing GSLs, are formed (Maccioni, 2007). The shortest of

the cancer associated glycolipids is GM3, which is has one sialic acid attached to the Lac-Cer structure (Birklé et al., 2003), and is highly up-regulated in brain, lung, and skin cancers (Zheng et al., 2019; Gu et al., 2008). GD2 and GD3 are longer glycans that are characterized by two sialic acids apiece and differ by an extra GalNAc structure on GD2 (Liu et al., 2018a). GD3 is a structure that is seen in low levels in healthy adults, and increases in GD3 correlate with a treatment resistant brain cancer stem cell population (Yeh et al., 2016). Elevated levels are seen in melanomas, acute lymphoblastic leukemias, and lung cancers, where it regulates tumor growth and proliferation (Furukawa et al., 2012; Merritt et al., 1994). Furthermore, the GD3 structure has been shown to bind and suppress both T helper and NKT cell function in brain and ovarian cancers (Mycko et al., 2014; Webb et al., 2012). GD2 is also a well-known ganglioside that is up-regulated in in melanoma, lung cancers, sarcomas, neuroblastomas, and triple-negative breast cancers (Dobrenkov et al., 2016; Orsi et al., 2017; Terzic et al., 2018; Yanagisawa et al., 2011). GD2 associates with the VLA-2 integrin and up-regulates the binding of neuroblastomas to ECM proteins such as collagens. In fact, anti-GD2 therapies (dinutuximab) are currently approved for use in the clinic to treat neuroblastomas and osteosarcomas (Greenwood and Foster, 2017; Roth et al., 2014).

Glycocalyx function in EMT, mechanics, and anti-tumor immunity

The glycocalyx plays a critical role in mediating the interactions between the tumor and its extracellular environment and has profound effects on EMT transition, mechanosensing, signaling, metastasis, and immune evasion, all of which will be discussed in the following sections.

Composition changes of the glycocalyx during EMT in cancer

The transition of cancer cells from an epithelial phenotype to a more mobile mesenchymal phenotype, the EMT, has in many instances been correlated with more invasive (Moustakas and de Herreros, 2017) and treatment-resistant cancers (Brabletz et al., 2018), and overall poorer patient outcomes (George et al., 2017). This a source of open debate, though, as not all cancers display EMT as a prerequisite to cancer metastasis and the link to human cancers in vivo is complex (Ledford, 2011). Even so, a major change associated with the EMT is the change to a more bulky and taller phenotype in the structure of the tumor glycocalyx (Lange-Consiglio et al., 2014; Mitchell and King, 2014; Paszek et al., 2014; Zeng et al., 2016).

For complex and hybrid N-glycans, a major change during the EMT is the loss of the bisecting GlcNAc structure and the accompanying up-regulation of β1,6 branches (Xu et al., 2017). The glycosyltransferase MGAT5 regulates the β1,6 branching, and studies in MGAT5-deficient breast tumors showed that ablation of MGAT5 and loss of β1,6 branching correlated with pronounced reductions in tumor growth and activation due to increased immune response from $CD4^+$ T cells and macrophages (Li et al., 2008). Furthermore, EMT in liver carcinoma (hepatocellular carcinoma) correlated with up-regulated core-fucose and β1,6 branching on N-glycans and sLe^x and T-antigens (Gal-GalNAc) on O-glycans (Li et al., 2013), all of which are markers of cancer as discussed in the previous section. MUC1 O-glycosylation is also specifically up-regulated during EMT in a variety of cancers (Freire-de-Lima, 2014). Deletion of the MUC1 cytoplasmic tail leads to a subsequent decrease in EMT and metastasis in pancreatic cancer through loss of its association with β-catenin, which is required for nuclear localization and transcription (Roy et al., 2011). Finally, glycolipid composition is also modified during EMT as sialylated gangliosides such as GM2, GD2, and GD3 are present at high levels in brain cancers while absent in normal tissues (Hakomori, 1996). More detailed information can be found in the comprehensive review of the changes seen in the glycocalyx during cancer EMT (Li et al., 2016).

Mechanosensing through the glycocalyx and its impact on integrins

The glycocalyx is the principal structure in contact with the ECM (Lahir, 2016); therefore, it plays a critical role in regulating both the adhesions of integrins to the ECM and the mechanics of the tumor cell in response to stiffness (Sun et al., 2016). Recent works were the first to demonstrate that the glycocalyx is intrinsically coupled to integrin clustering and mechanosensing with the ECM (Paszek et al., 2009, 2014). Initially, a computational model made two main predictions: first, that cell-bound integrins cluster and bind to the ECM more avidly with increasing stiffness, and, second, that the size and bulk of the glycocalyx limits the ability of integrins to cluster and bind due to the intrinsic repulsion between the glycocalyx and the ECM. According to this model, the composition and size of the cellular glycocalyx modulate the degree of mechanosensing that cell-bound integrins undergo when in contact with the ECM (Paszek et al., 2009). A second study experimentally examined this computational model, demonstrating that in cancer, the glycocalyx and especially bulky cancer-related glycoproteins such as MUC1, drive integrin clustering and mechanosensing. The overall mechanism involves bulky glycoproteins acting as physical barriers that funnel integrins to cluster in the adhesive zones in contact with the ECM. Finally, the bulky glycoproteins are able to reinforce or enhance mechanosensing, and this in turn promotes cancer cell growth and survival (Paszek et al., 2014).

The role of the glycocalyx in cancer progression was expanded by recent studies demonstrating that a bulky glycocalyx drives the metastatic potential by increasing cell cycle progression through the phosphoinositide 3-kinase–AKT axis and mechanosensing through integrin-FAK interplay (Woods et al., 2017). Furthermore, increasing the size of the glycocalyx with the MUC1 ecto-domain was sufficient to drive metastatic potential in an in vivo model of breast cancer (Woods et al., 2017). A subsequent study found that in glioblastoma multiforme, a mesenchymal phenotype is linked with greater aggression, and this is regulated by increased mechanosensing through stiffer ECM substrates (Barnes et al., 2018). Overall, a bulkier glycocalyx is linked with a more mesenchymal and aggressive cancer phenotype, and mechanosensing up-regulates both mesenchymal and bulky glycocalyx-related genes, most principally galectin-1, to drive aggression (Barnes et al., 2018). This work was the first to describe a tension-mediated glycocalyx–integrin

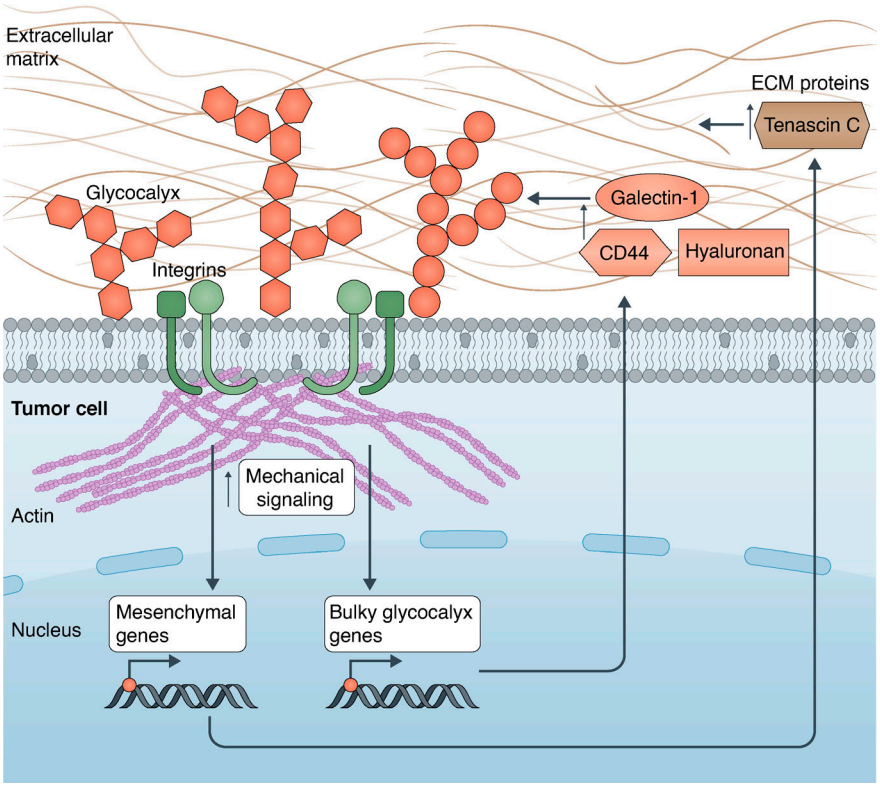

Figure 3. **Tension mediates a glycocalyx-integrin feedback loop in cancer.** The glycocalyx drives enhanced mechanical signaling between the integrin–actin axis on the tumor cell and the ECM. This mechanical signaling promotes the up-regulation of genes in the nucleus driving both a more mesenchymal phenotype (production of ECM proteins such as Tenascin C) and a bulkier glycocalyx (such as CD44, hyaluronic acid, and galectin-1) in the tumor. Together, these drive glioma aggressiveness in a tension-dependent feedback loop, which is self-enforcing. Illustration by Neil Smith (http://www.neilsmithillustration.co.uk).

feedback loop wherein mechanical signaling through the FAK pathway up-regulates both mesenchymal and bulky glycocalyx genes, which in turn increases the glycocalyx size and further enhances integrin mechanosignaling (Fig. 3). Knockdown of either the glycocalyx component galectin-1 or FAK was able to decrease both glycocalyx bulkiness and the glioblastoma multiforme aggressiveness (Barnes et al., 2018).

The role of the glycocalyx in promoting migration, invasion, and metastasis

A larger and bulkier glycocalyx has been implicated in both increased migration and metastasis during cancer (Lahir, 2016). As discussed, a taller, bulkier glycocalyx is associated with both increased migration (Barnes et al., 2018) and metastatic potential of cancers (Woods et al., 2017). Furthermore, the cancer cell glycocalyx itself acts as a mechanosensor that responds to the interstitial flow coming from the comprised tumor vasculature, which can increase the migration and invasion of the tumor (Tarbell and Pahakis, 2006; Tarbell and Shi, 2013; Zeng and Tarbell, 2014). The glycocalyx responds to the mechanical force generated by the shear flow by secreting matrix metalloproteinases into the ECM to degrade it. This promotes tumor migration, as the degraded ECM is easier to move through, and eventual movement of the tumor out of the tissue and into the vasculature to colonize secondary sites (Qazi et al., 2011, 2013, 2016). The ability of the glycocalyx to act as a mechanosensor to degrade the ECM holds therapeutic potential as manipulating the glycocalyx structure could influence the mechanical response to fluid stress (Tarbell and Cancel, 2016). Furthermore, it could impact whether tumor cells can migrate against the direction of shear flow to reach the vasculature, a phenomenon so far only seen in various immune cell subsets that allows for faster and more effective trans-endothelial migration (Anderson et al., 2019; Buffone et al., 2018, 2019; Dominguez et al., 2015; Tedford et al., 2017; Valignat et al., 2013).

Sialic acid shielding evades anti-tumor immunity

The final major way in which the cancer cell glycocalyx is known to affect tumor progression is by shielding the tumor from immune surveillance and the subsequent immune response (Kim et al., 2007; Xiao et al., 2016). The tumor glycocalyx accomplishes this by tricking the immune system into thinking the tumor is part of its normal, healthy tissue via an increase in terminal sialic acids (Büll et al., 2014; Varki and Gagneux, 2012). High sialic acid content of the cancer glycocalyx has been implicated in both decreased anti-tumor activity and poorer survival outcomes (Brossart et al., 2001). Perhaps most critically, the increased sialic acid content in tumor cells increases the amount of ligands for self-inhibitory siglecs on the immune cell surface (Crocker et al., 2007). Increased sialylated glycans "trick" the responding NK cell into thinking the tumor is healthy tissue by activating both Siglec-7 and -9 (Avril et al., 2004; Hudak et al., 2014; Jandus et al., 2014). Blocking the function of Siglec-7 or -9 with antibodies or removing the terminal sialic acids from the tumor has been shown to restore NK-mediated tumor killing (Jandus et al., 2014; Nicoll et al., 2003).

To conclude, the specific content and structure of the cancer cell glycocalyx has a critical role in mediating all aspects of tumor fate, from mechanosensing, signaling, migration, and metastasis to immune evasion. To this end, in order to realize the full therapeutic potential of manipulating the cancer cell glycocalyx, precise editing of the glycocalyx at the monosaccharide

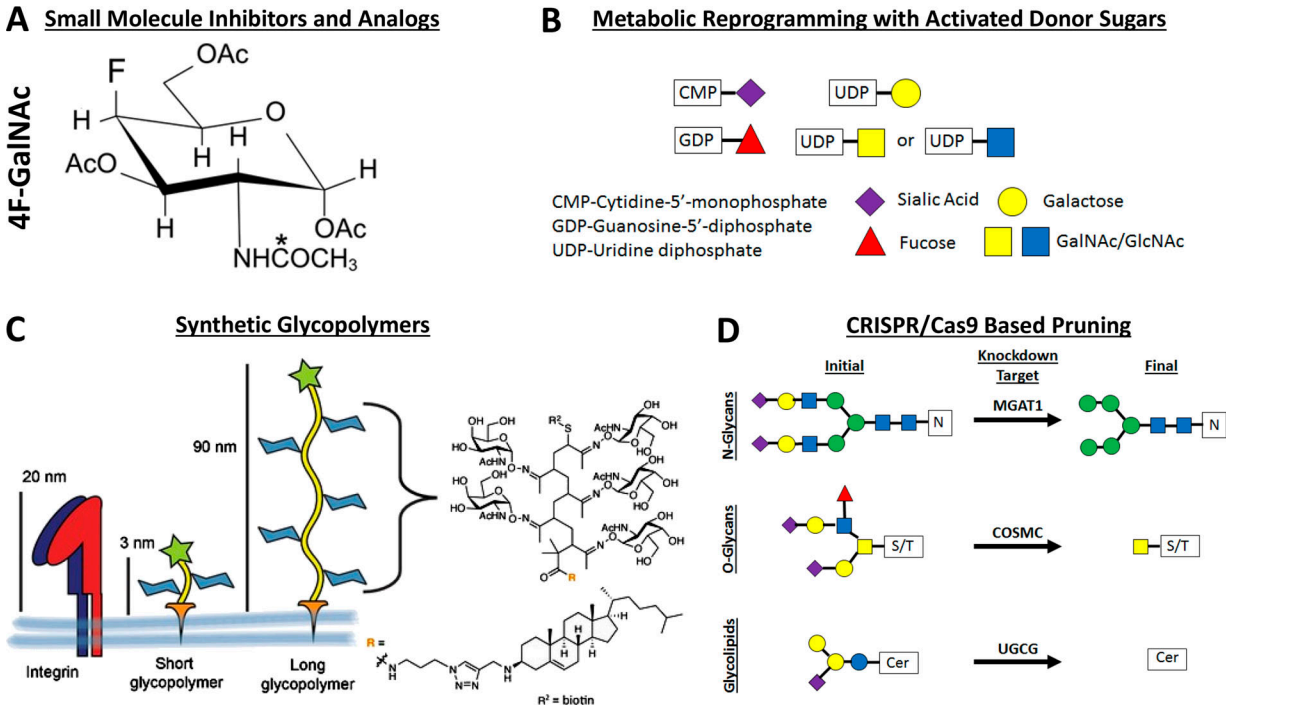

Figure 4. **Methods for editing the cancer glycocalyx. (A–C)** Representation of the various methods used to edit the cancer glycocalyx including small molecule analogues such as 4F-GalNAc, which incorporate into and truncate glycan chains (A); addition of the activated sugar donors to cells to metabolically reprogram glycan synthesis by manipulating the amount of substrate for glycosyltransferases (B), and synthetic glycopolymers that attach to the cell membrane and can change the height of the glycocalyx (C), which affects integrin binding and clustering. The asterisk denotes the acetylation site. **(D)** Genetic knockdown of MGAT1, COSMC, or UGCG can precisely truncate N-glycan, O-glycan, and ganglioside biosynthesis. Panel C was adapted from Woods et al. (2017).

level is needed. In the final section, we will discuss the currently used methods for editing the glycocalyx, and potential strategies to prune the specific glycans of the glycocalyx as if it were a tree.

Tools for specifically pruning the glycocalyx

As discussed, the structure, content, and bulkiness of the glycocalyx have profound effects on the mechanics surrounding tumor growth and signaling, along with immune evasion and EMT. What is lacking are precise tools to specifically manipulate the structure of the glycocalyx. A variety of approaches have been attempted to manipulate the glycans; however, they still lack the precision needed to make specific alterations.

Small molecule inhibitors and mimetics

The basic premise of small molecule inhibitors is to design them in such a way that they either incorporate into the native glycan during synthesis to stop further elongation of the glycan or act as a natural competition for the glycan recognition site to stop the binding and signaling processes associated with the glycan (Jacob, 1995; Esko and Schnaar, 2017). In terms of the incorporation of a nonnative sugar, these act mainly as metabolic decoys that hijack native glycosyltransferase activity and incorporate themselves into the native glycan trees (Fig. 4 A). To this effect, many different synthetic sugars have been generated to reprogram N- and O-glycan biosynthesis, including analogues to sialic acids (Macauley et al., 2014; van den Bijgaart et al., 2019), fucoses (Okeley et al., 2013), GalNAcs (Marathe et al., 2010), GlcNAcs (Barthel et al., 2011; Gainers et al., 2007), and xylose (Garud et al., 2008). Furthermore, inhibitors have been developed to modify other major glycosylation pathways including O-GlcNAc pathways in mucin biosynthesis (Liu et al., 2018b) and glycolipids (Nimrichter et al., 2008). The drawback to these inhibitors is that most have minimal incorporation into cells at relatively high millimolar concentrations, which limits their therapeutic potential in the clinic (Kudelka et al., 2016). Some newly described glycosylation mimetics such as thioglycosides (Wang et al., 2018) may be able to overcome the hurdle of how to get high incorporation at lower concentrations.

On the other hand, several glycan mimetics that compete away binding activity of the native glycan receptors have found great promise as therapeutics and are either on their way to or in the clinic. Mimetics against the sLe^x tetrasaccharide along with other ligands have been used in clinical trials for pan selectin inhibition (Chang et al., 2010; Morikis et al., 2017) to treat sickle cell crises, E-selectin inhibition to treat acute myeloid leukemia in bone marrow (Winkler et al., 2012), and combined E-selectin and CXCR4 inhibition (Price et al., 2016) to treat dormant breast cancer. Furthermore, glycan-based therapeutics to cleave sialic acid ligands on cancer cells to prevent siglec-based shielding from immunosurveilance are also entering clinical trials (Haas et al., 2019; Stanczak et al., 2018). While small molecule inhibitors and mimetics have therapeutic applications, they still represent a brute force approach to manipulating glycosylation and lack the precision needed to specifically edit the glycocalyx.

Metabolic reprogramming of sugar donors

Another method of editing the content and structures of the glycocalyx includes manipulating the metabolic pathways involved in the synthesis of the activated sugar donors needed for glycoyltransferases to catalyze their addition to growing glycan chains (Fig. 4 B). This includes the activated sugars CMP-Neu5Ac, GDP-Fuc, and UDP-GlcNAc. Loss of the CMP–sialic acid transporter (Martinez-Duncker et al., 2005) and the GDP-fucose transporter, called leukocyte adhesion deficiency II (Lübke et al., 2001), are both congenital disorders of glycosylation seen in humans, and are characterized by defects in the SLC35A1 and SLC35C1 genes, respectively. Researchers have used knowledge from these disorders to disruptively manipulate the sialylation and fucosylation patterns of cancer cells. Manipulation of the amount of GlcNAc has been done both by feeding it into the diet of mice or adding it exogenously to PymT tumor cells to up-regulate UDP-GlcNAc and rescue the branching of their N-glycans in the absence of MGAT5. This in turn worsens their cancer prognosis as the more complex N-glycans correlate with more growth factor signaling and galectin-3 binding (Mendelsohn et al., 2007; Ryczko et al., 2016). Another major way that the metabolic pathways involved in glycosylation have been modified is the use of tunicamycin to block proper N-glycosylation (Merlie et al., 1982). Tunicamycin inhibits GlcNAc phosphotransferase, which transfers GlcNAc-1-phosphoate to dolichol phosphate, during the first steps of N-glycan synthesis (Wyszynski et al., 2012). Thus, tunicamycin has been used as a chemical tool in research as a way to study the effect of disrupted N-glycosylation. In sum, while metabolic regulation of the donor sugars is a viable mechanism to modify the glycocalyx, it lacks the level of specificity needed to manipulate the cancer glycocalyx at the single-sugar level.

Modifying the protein backbone with recombinant and synthetic glycoproteins

Another viable approach to modifying the structure of the glycocalyx has involved modifying the protein and lipid backbones that carry the glycans themselves (Fig. 4 C). For example, modification of both the height and bulk of the glycocalyx through the use of mucin mutants of varying lengths has been accomplished to tune the response to stiffness in cancer (Barnes et al., 2018; Kramer et al., 2015; Paszek et al., 2014; Woods et al., 2017). Modified glycoproteins that tune glycosylation have also been used as a way to enforce selectin-binding activity in mesenchymal stem cells (Abdi et al., 2015; Lo et al., 2013a, 2016), to present antigens to activate dendritic cells (García-Vallejo et al., 2013), and to increase NK-mediated killing of cancer cells by competing away siglec binding (Hudak et al., 2014). Again, while these methods are elegant in their control of the protein backbone, they are only able to control the glycosylation by loss or gain of potential glycosylation sites. This leaves manipulating glycan structures at the single sugar level out of the scope of these tools.

CRISPR-based pruning of specific glycosyltransferases

While the other approaches discussed here rely on bulk disruption of the glycan structures by incorporation of a modified sugar, removing or increasing the activated donor sugar, or actually manipulating the protein or lipid backbone, disruption of the glycosyltransferase enzyme associated with the addition of a specific monosaccharide represents an elegant method for the specific truncation of glycan structures (Steentoft et al., 2014). This approach is two-pronged as it can be used to either remove the enzymatic function of a specific glycan (Buffone et al., 2013; Mondal et al., 2015) or target the chain-initiating enzyme of the entire glycan tree (Mondal et al., 2016; Vester-Christensen et al., 2013). In fact, specific removal of N-glycosylation has been used as a clinical tool for anti–PD-L1 antibodies to more robustly recognize PD-L1 and enhance treatment of breast cancer (Lee et al., 2019). Two recent toolkits have been developed in order to specifically manipulate the composition and structure of the glycocalyx. The first is a comprehensive toolkit for manipulating the characteristics of the O-glycome on cancer cells at both the glycan and protein backbone levels (Shurer et al., 2018). This toolkit has allowed them to elegantly quantify the biophysics of the glycocalyx and manipulate its shape and function (Shurer et al., 2019). The second toolkit allows for the elegant quantification of the relative function of specific glycan classes (Stolfa et al., 2016). The knockdown of MGAT1, COSMC, and UGCG to specifically truncate complex and hybrid N-linked glycoproteins, O-linked glycoproteins, and glycolipids can precisely quantity the relative function of each type of glycan (Fig. 4 D). Although this was applied in the context of leukocyte recruitment to the sites of inflammation (Stolfa et al., 2016), this toolkit holds great potential in a wide variety of settings, most notably in determining the critical glycans regulating the size and bulk of the glycocalyx, and in turn the mechanics, in cancer.

Conclusions and future outlook

To summarize, the tumor cell glycocalyx is critical in regulating tumor cell–ECM mechanosensing as it acts as a buffer for interactions between the cell surface receptors and surrounding tissues. The glycocalyx composition affects all aspects of tumor cell progression including cellular tension, integrin signaling, migration, metastasis, and immune recognition and also drives a feedback loop to increase the height and bulk of the glycocalyx and sustain the pro-cancer phenotype. While de-bulking the glycocalyx through the use of synthetic mucins can disrupt this cancer feedback loop by attenuating mechanical signaling, better tools are needed to prune the glycocalyx in a more precise manner. Being able to quantify the relative contribution of the N-linked, O-linked, and glycolipid components of the glycocalyx represents a significant step forward in attributing a specific glycan moiety to regulation of the tension-feedback loop. With this said, in order to truly unlock the potential of controlling the glycocalyx-integrin mechanosensing feedback loop to halt cancer progression, tools must developed to prune the glycans at a single-sugar level.

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