THE SWEET AND SOUR OF CANCER: GLYCANS AS NOVEL THERAPEUTIC TARGETS

Mark M. Fuster and Jeffrey D. Esko‡*

Abstract | A growing body of evidence supports crucial roles for glycans at various pathophysiological steps of tumour progression. Glycans regulate tumour proliferation, invasion, haematogenous metastasis and angiogenesis, and increased understanding of these roles sets the stage for developing pharmaceutical agents that target these molecules. Such novel agents might be used alone or in combination with operative and/or chemoradiation strategies for treating cancer.

GLYCOCONJUGATE
A molecule in which one or more glycan units are covalently linked to a non-carbohydrate entity.

N-LINKED GLYCANS
Glycans covalently linked to an asparagine residue of a polypeptide chain in the consensus sequence –Asn–X–Ser/Thr.

GLYCOPROTEIN
A protein with one or more covalently bound glycans.

Department of Medicine, Division of Pulmonary and Critical Care Medicine, University of California, San Diego, La Jolla, California, 92093-0687, USA.
‡Department of Cellular and Molecular Medicine, Glycobiology Research and Training Center, University of California, San Diego, La Jolla, California, 92093-0687, USA.
Correspondence to J.D.E.
e-mail: jesko@ucsd.edu
doi:10.1038/nrc1649

Several glycans, on both the tumour surface and host elements, have now been identified as mediating key pathophysiological events during the various steps of tumour progression. Tumour progression involves a range of unique alterations in intracellular and intercellular signalling. These serve to promote dysregulation of the cell cycle and facilitate proliferation; to promote the emergence of a subset of invasive cells that dissociate from the tumour and digest and migrate through host extracellular matrix (ECM) and basement membranes; to summon an endothelial-lined neovascular network from nearby host endothelial cells (angiogenesis); to endow disseminating tumour cells with cell-surface characteristics that promote adhesive interactions with platelets, leukocytes and blood or lymphatic vascular endothelial cells; and to facilitate the evasion of innate immunity. Ultimately, a 'survivor' subset of cells must invade, neovascularize, disseminate, extravasate and proliferate at a new tissue location to become a pathological metastasis.

Glycans are covalent assemblies of sugars (oligosaccharides and polysaccharides) that exist in either free form or in covalent complexes with proteins or lipids (GLYCOCONJUGATES). There are several main families of glycoconjugates: the Asn-linked (N-LINKED) oligosaccharides of many GLYCOPROTEINS; the Ser- or Thr-linked (O-LINKED) oligosaccharides that are present on many glycoproteins and that predominate on secreted and membrane bound MUCINS; the glycosaminoglycans, which are glycans present as free polysaccharides (such as hyaluronan) or as part of PROTEOGLYCANS (such as heparan sulphate and chondroitin sulphate); the glycosphingolipids, which consist of oligosaccharides glycosidically linked to ceramide; the glycosylphosphatidylinositol (GPI)-linked proteins, which are proteins that bear a glycan chain linked to phosphatidylinositol; and nuclear and cytoplasmic proteins, which bear the monosaccharide *O*-linked *N*-acetylglucosamine (*O*-GlcNAc) linked to serine, often at sites that are normally phosphorylated[1] (FIG. 1). Most classes of glycan exist as membrane-bound glycoconjugates (for example, in the GLYCOCALYX) or as secreted molecules, which can become integral parts of the ECM. These locations place glycans in a position to mediate cell adhesion and motility, as well as intracellular signalling events[2].

In the tumour environment, changes in glycosylation allow neoplastic cells to usurp many of the events that occur in development (for example, receptor activation, cell adhesion and cell motility), which allows tumour cells to invade and spread throughout the organism[3]. Malignant transformation is often accompanied by the expression of oncofetal antigens — epitopes that are expressed on embryonic tissues and tumour cells, and only in a few cell types in the adult. Many of the first-identified tumour-specific antibodies were directed

© 2005 Nature Publishing Group

Summary

- Tumours aberrantly express various glycans. Glycans regulate many different aspects of tumour progression, including proliferation, invasion, angiogenesis and metastasis.
- The proliferation of tumour cells is potentiated by the ability of glycoproteins and glycosphingolipids to directly activate growth-factor receptor tyrosine kinases and by the ability of proteoglycans to function as co-receptors for soluble tumour growth factors.
- The overexpression of specific glycosyltransferases by tumour cells promotes the formation of tumour glycans that facilitate invasion.
- Carcinomas commonly overexpress *O*-linked glycans in the form of cell-surface and secreted mucins that present ligands for adhesion receptors, such as the selectins, which promote the ability of tumour cells to interact with host platelets, leukocytes and endothelial cells. These interactions facilitate haematogenous metastasis of tumour cells.
- Glycosphingolipids, in the form of gangliosides, are overexpressed by a range of tumours, and their shedding into the bloodstream might impair host immunity to some tumours.
- During tumour proliferation and invasion, heparan-sulphate proteoglycans (HSPGs) that are present on the surface of tumour cells function as co-receptors to stabilize growth-factor receptor signalling complexes. Secreted HSPGs that are present in the extracellular matrix store growth factors that can be mobilized by the action of tumour heparanases. A similar mechanism that involves endothelial-associated HSPGs and endothelial growth factors facilitates vascular sprouting during tumour angiogenesis.
- Some glycans can be measured in the bloodstream, and their use as markers of disease burden can be used to screen for specific cancers as well as track response to therapy.
- Experiments in which glycan function is genetically altered in cell-culture systems or mouse tumour models validate their potential as targets for anticancer therapy.
- A few glycan-based targeting strategies are currently being tested in clinical trials. As we learn more about the roles of glycans in tumour progression, new targets will continue to emerge for drug design.

against carbohydrate oncofetal antigens presented on tumour glycoproteins and glycosphingolipids[3,4]. In some cases, the underexpression, truncation or altered branching patterns of certain glycans correlate with cell growth. Similar alterations on tumours endow them with enhanced proliferative capacity, which could reflect the outcome of 'Darwinian selection' of rapidly growing cells that can endure survival pressures imposed by the host. A massive potential for glycan diversity exists, but a relatively limited array of glycans correlates with invasion and metastatic potential across a wide range of tumours.

Initial insight into the unique repertoire of glycans expressed on tumour cells emerged from the increased ability of tumours to bind a range of plant LECTINS[5]. Lectins exhibit protein folds that define families of carbohydrate-binding proteins that can bind in a specific 'lock-and-key' fashion[6]. Various endogenous animal lectins also exist, and these facilitate fundamental processes such as quality control of secreted proteins, cell–cell recognition, cell adhesion and motility, and pathogen–host recognition. Many lectins exist on the surface of immune cells and endothelial cells that line the vasculature, as ECM proteins and as soluble adhesion molecules such as mannose-binding proteins or galactose-binding lectins (galectins), and many of these can associate with tumour-cell-associated glycans. The interactions of lectins with tumour-cell glycans facilitate all aspects of tumour progression.

This article will mainly focus on examples in which genetic or pharmacological data confirm a correlation between a specific pattern of glycan expression and tumour progression. We have organized this review according to the main stages of tumour progression: proliferation, invasion, angiogenesis, metastasis and immunity (FIG. 2). We refer readers interested in more details to several excellent reviews on specific glycan classes and cancer[3,7–13].

Proliferation of tumour cells

Tumour cells arise from mutations in proto-oncogenes and tumour suppressors that normally control the cell cycle and affect DNA repair[14]. Tumours often express a unique repertoire of glycans. These changes might reflect selection of cell clones that have distinct glycan compositions, but correlating these changes to proliferation itself has been difficult. Nevertheless, a few examples illustrate how alterations in protein or lipid glycosylation can stimulate cell growth (TABLE 1).

Effects on tumour growth-factor receptors. *N*-linked glycans have important roles in protein folding, quality control and half-life; cell–cell recognition and adhesion; and signalling[15] (FIG. 1). Because *N*-glycosylation of glycoproteins is so prevalent, the changes in *N*-glycan biosynthesis can have pleiotropic effects on many systems, making it difficult to correlate tumour growth with a change in glycosylation of a specific protein. For example, *N*-glycosylation of the insulin-like growth factor 1 receptor (IGF1R) is required for IGF1R phosphorylation, cell-surface translocation, and the subsequent growth and survival of melanoma and sarcoma cells[16]. Treating cells with *N*-glycosylation inhibitors *in vitro* can inhibit the survival of tumours that depend on signalling by IGF1R.

Extensive literature exists about the roles of *O*-linked glycans and mucins in proliferation (FIG. 1). Mucins are glycoproteins that contain numerous *O*-glycans in clustered domains along the core protein. The MUC gene superfamily (reviewed in REF. 12) is a family of mucin core proteins that are expressed in a tissue-specific manner and serve as markers for epithelial cells. Most carcinomas express mucins, either as transmembrane proteins on the cell surface or as secreted proteins. MUC expression often goes awry in the tumour. For example, mammary tumours overexpress MUC4 on the cell surface[17], whereas it is not expressed on the normal mammary epithelium. MUC4 contains an epidermal growth factor (EGF)-like motif on its extracellular (juxtamembrane) domain that directly interacts with ERBB2 (a member of the EGF-RECEPTOR FAMILY), initiating phosphorylation of the receptor tyrosine kinase in the absence of more typical ERBB ligands (such as EGF)[12]. ERBB2 is frequently overexpressed (or mutated to the activated state) in breast carcinomas.

O-LINKED GLYCANS
Glycans glycosidically linked to the hydroxyl group of the amino acids serine, threonine, tyrosine or hydroxylysine.

MUCINS
Large glycoproteins with a high content of serine, threonine and proline residues, and numerous *O*-linked glycans, often occurring in clusters on the polypeptide. Tumour mucins are often decorated by unique small glycans such as Tn (*O*-linked GalNAc) or sialyl Tn antigens (sialic acid-capped *O*-linked GalNAc).

© 2005 Nature Publishing Group

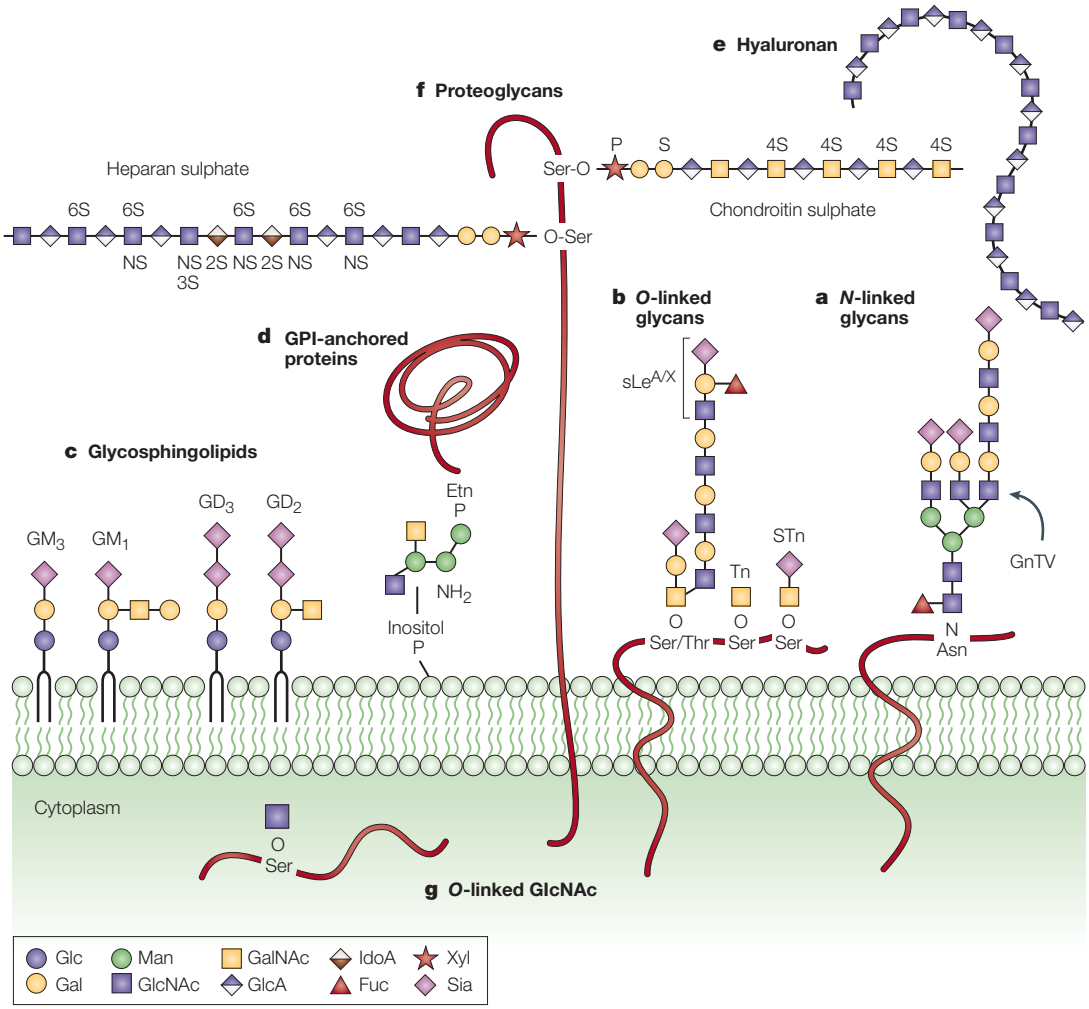


Figure 1 | **Important glycans involved in tumour progression.** Unique glycans are involved in promoting the progression of various carcinomas. **a** | *N*-linked glycans on glycoproteins are covalently bound to Asn residues. Typical branched structures contain two or more 'antennae'. The enzyme *N*-acetylglucosaninyltransferase V (GnTV) generates a specific antenna on some glycoproteins and has been implicated in tumour invasion. **b** | *O*-linked glycans are found covalently linked to Ser or Thr residues on glycoproteins and mucins. $SLe^{X/A}$ (sialyl Lewis X or A) are carbohydrate determinants composed of four sugars in specific linkage to one another, and are commonly overexpressed on tumour-cell mucins. The determinant forms part of a ligand for the selectin class of adhesion receptors involved in tumour-cell aggregation with leukocytes and platelets, and adhesion of tumour cells to endothelial cells. Tn and STn are tumour antigens that consist of truncated *O*-linked chains. Their accumulation in many tumours correlates with invasion. **c** | Glycosphingolipids consist of the lipid ceramide linked to one or more sugars. Certain sialic-acid-containing glycosphingolipids, called gangliosides (for example, GM_1, GM_3, GD_2 and GD_3), have been correlated with tumour growth. **d** | Glycosylphosphatidylinositol (GPI)-linked proteins are anchored in the outer leaflet of the plasma membrane by a glycan covalently linked to phosphatidylinositol. Glycosaminoglycans can occur as free chains (hyaluronan; **e**) or as covalent complexes with proteoglycan core proteins (heparan sulphate, chondroitin sulphate and dermatan sulphate, a type of chondroitin-sulphate-containing iduronic acid (IdoA)). **f** | Proteoglycans participate in growth-factor activation and cell adhesion. **g** | Various cytoplasmic and nuclear proteins contain *O*-linked *N*-acetylglucosamine (*O*-GlcNAc). Some glycoconjugates can be tethered to the plasma membrane as depicted or secreted into the extracellular matrix. In some cases, hybrid molecules exist, containing more than one type of glycan. Sugars are represented by coloured geometric symbols. Glc, glucose; Gal, galactose; Man, mannose; GlcNAc, *N*-acetylglucosamine; GalNAc, *N*-acetylgalactosamine; GlcA, glucuronic acid; Fuc, fucose; Xyl, xylose; Sia, sialic acid.

PROTEOGLYCAN
A protein with one or more covalently attached glycosaminoglycan chains, such as heparan sulphate or chondroitin sulphate (and dermatan sulphate). In the tumour environment, a range of heparan-sulphate proteoglycans expressed by both tumour cells as well as endothelial cells affect growth-factor signalling.

GLYCOCALYX
The cell-coat structure consisting of glycans and glycoconjugates surrounding animal cells. This is seen as an electron-dense layer by electron microscopy.

LECTIN
A protein (other than an anti-carbohydrate antibody) that specifically recognizes and binds to glycans without catalysing a modification of the glycan.

EGF-RECEPTOR FAMILY
Epidermal growth factor receptors have important roles in initiating the signalling that directs the behaviour of epithelial cells and tumours of epithelial origin. The four members of the family are also known as ERBB receptor tyrosine kinases (ERBB1–4), and share structural and functional similarities.

GANGLIOSIDES
Anionic glycosphingolipids containing one or more residues of sialic acid.

Recent experiments show that the overexpression of MUC4 in melanoma cells induces rapid cellular growth and the suppression of tumour apoptosis in human melanoma-bearing nude mice[18]. Furthermore, MUC4-overexpressing cells can autophosphorylate ERBB2, which contributes to inhibition of apoptosis[18]. The specific role of *O*-glycosylation in this process is unknown. The glycan moiety might affect MUC4 protein folding or exposure of the EGF-like domains on the MUC4 backbone in a manner that promotes interaction with ERBB2.

Many tumours overexpress glycosphingolipids, especially GANGLIOSIDES, which are 'capped' with sialic acid (a negatively charged acidic sugar) on the outermost tip of the glycan (FIG. 1). In normal cells, gangliosides are often associated with various receptor

© 2005 Nature Publishing Group

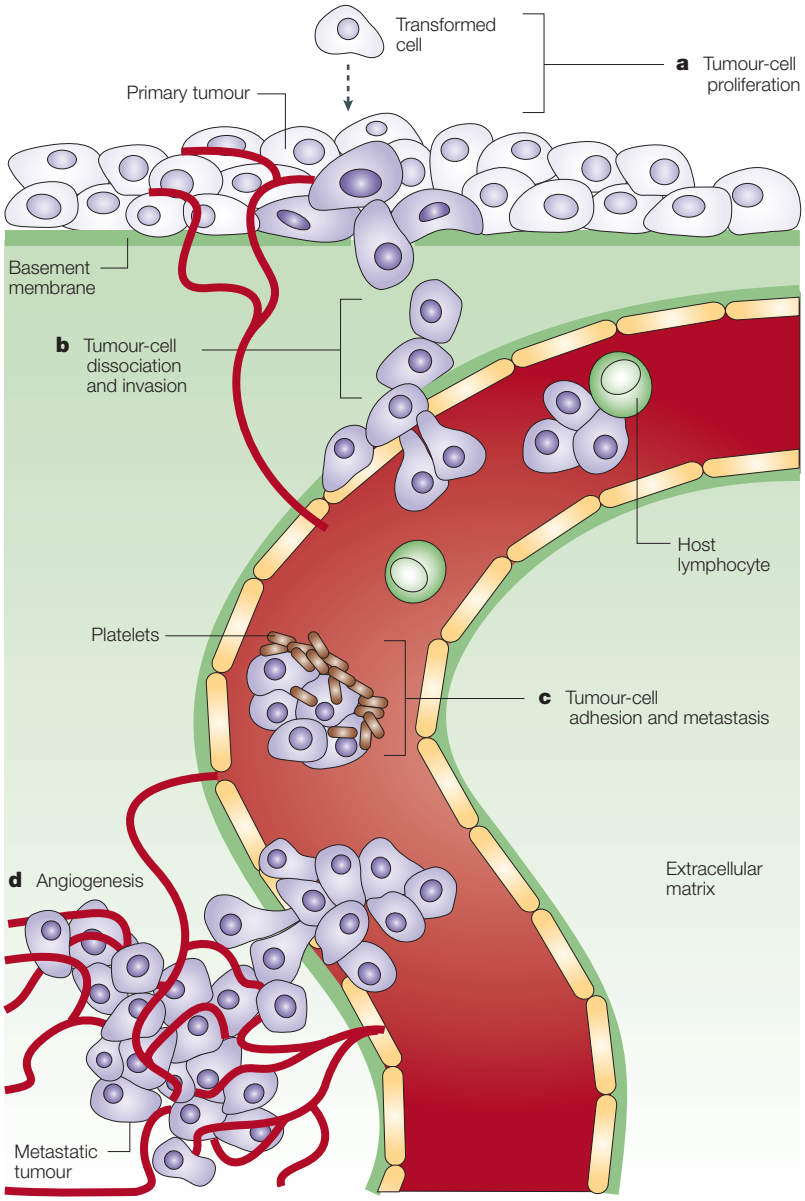


Figure 2 | **Stages of tumour progression.** Tumour proliferation (**a**) is crucial at early stages of progression. **b** | During invasion, tumour cells gain the capacity to degrade, and migrate through, basement membranes and extracellular matrix. **c** | During the dissemination of tumour cells through the bloodstream, they aggregate with host cells such as platelets and lymphocytes and eventually lodge in the small vessels of distant organs. **d** | Tumour angiogenesis is required for pathological growth of the primary cancer and its metastases. Glycans have roles in each of these stages of tumour progression. See FIG. 3 for more details.

LIPID RAFTS
Microdomains in the plasma membrane that are enriched in sphingolipids, cholesterol and GPI-linked proteins. They function as signalling platforms through their ability to concentrate signalling proteins, resulting in increased output from receptors that require cross-activating interactions and increasing local concentrations of other downstream signalling components.

tyrosine kinases (such as EGF or insulin receptors) and modulate their phosphorylation. Evidence indicates that the interaction between gangliosides and receptor tyrosine kinases serves important growth-promoting functions. For example, the gangliosides G_{M3} or G_{D3} — which are commonly overexpressed in lung cancers, melanomas and neurogenic tumours — are able to regulate growth signalling through interactions with membrane-associated receptor tyrosine kinases or protein kinase C[3,8,19]. At a higher level of molecular organization, gangliosides on breast cancer cell membranes regulate the formation of EGF receptor complexes in LIPID RAFTS (FIG. 3). In particular, the ganglioside G_{M1} is necessary for ERBB2 and ERBB3 receptors to form growth-factor-responsive heterodimers on lipid rafts[20], thereby facilitating ERBB signalling. The ERBB receptors provide an example in which the surrounding milieu of glycans (in this case glycosphingolipids) and a specific protein–glycoprotein (in this case MUC4) interaction can modulate the function of a crucial growth receptor.

Glycans as co-receptors for soluble tumour growth factors. Proteoglycans contain a core protein and one or more covalently attached glycosaminoglycan (GAG) side chains (FIG. 1). Unlike *N*- or *O*-linked glycans, GAGs are composed of repeating disaccharide units that are composed of an amino sugar (GlcNAc in heparan sulphate or GalNAc in chondroitin sulphate; see FIG. 1) linked to a uronic acid (GlcA or IdoA). Negatively charged sulphate groups at discrete positions along heparan-sulphate chains facilitate interactions with basic amino-acid residues on a range of protein ligands involved in cell–matrix interactions (for example, laminin, fibronectin and thrombospondin), inflammation (for example, the P and L SELECTINS) and growth (for example, the fibroblast growth factors (FGFs), vascular endothelial growth factor (VEGF), transforming growth factor-β (TGFβ) and interleukin-8 (IL-8))[21–23]. GAGs are able to facilitate the formation of ligand–receptor complexes, lowering the effective concentration of ligand required for receptor activation. In this way, GAGs act as 'co-receptors'. GAGs also facilitate the storage of ligands for future mobilization and the protection of ligands from degradation[21,22]. These functions are usurped in the tumour environment, allowing tumour-released growth factors to exert autocrine effects as well as paracrine effects on surrounding host cells, such as endothelial cells that also bear growth-factor receptors.

The proliferation of tumour cells depends on heparan-sulphate proteoglycans (HSPGs). For example, glypican-1 (a GPI-anchored HSPG; FIG. 1) is overproduced by pancreatic cancer cells, and mediates mitogenic responses by tumour cells to basic fibroblast growth factor (FGF2) and heparin-binding EGF-like growth factor (HB-EGF) by facilitating the formation of ligand–receptor complexes[24]. This interaction occurs at specific sulphate-modified domains along cell-surface heparan-sulphate chains[22]. In pancreatic, breast, ovarian and hepatocellular cancers, tumour cells regulate genes that modulate sulfation of cell-surface HSPGs in a manner that increases their binding capacity for growth-factors and the activation of receptor tyrosine kinases[25]. Other examples are provided in TABLE 2.

In some cells, HSPGs can have the opposite effect, acting as tumour suppressors. For example, in Simpson–Golabi–Behmel syndrome, deletions or point mutations within the glypican-3 gene cause a congenital overgrowth syndrome, and patients have an increased risk of developing certain malignancies[26]. It is unclear how glypican-3 modulates growth, but it appears to modify epithelial responses to growth

© 2005 Nature Publishing Group

Table 1 | **Examples of glycan families involved in tumour progression**

Glycan involved	Proposed major function(s)	Possible therapeutic targeting	Examples of neoplasms	References
Growth and proliferation				
N-glycans	Suppress apoptosis; growth-factor signalling	Alkaloid inhibitors of N-linked processing	Breast, melanoma, Ewing's sarcoma	16,18
O-glycans	Mucin (MUC4)-mediated activation of ERBB2 receptors	Immunotherapy targeting MUC4 (similar to other mucin-targeting immunotherapy)	Breast	18
O-glycans	Suppress apoptosis (possibly through galectin-3 binding to tumour *O*-glycans expressing terminal galactose)	Galectin-3 inhibitors (β-galactosides)	Colon, pancreatic	150
Glycosphingolipids	Control of signalling through lipid rafts	Ceramide glycosylation inhibitors; ganglioside-targeted vaccines	Breast	20
Heparan-sulphate proteoglycans	Coreceptors for tumour growth factors	Heparin derivatives as heparan-sulphate competitors; sulphotransferase inhibitors	Pancreatic, ovarian, renal, hepatic	24,25
Hyaluronan	Signaling through hyaluronan receptors (for example, CD44)	Hyaluronan oligomers; adenoviral delivery of hyaluronan-binding protein genes	Colon, breast	13,30
O-GlcNAc	Modify oncogene phosphorylation	*O*-GlcNAc transferase inhibitors	Pancreatic	31
Tumour invasion				
N-glycans	Alter E-cadherin-dependent tumour adhesion	Alkaloid inhibitors of *N*-glycan processing	Breast, colon	41,74
N-glycans	Tumour repulsion (for example, polysialylation)	Sialyltransferase inhibitors	Neuroblastoma, lung (small cell)	43
O-glycans	Stimulate migration; potentiate migration of tumour cells through inhibition of cell–cell contacts (for example, sialyl Tn on mucins)	Vaccines (for example, conjugated sialyl Tn)	Breast, gastric, ovarian	47
Glycosphingolipids	Tumour repulsion (for example, G_{M3})	Glycosphingolipid inhibitors; ganglioside-targeted vaccines	Melanoma, neuroblastoma, breast	35,36, 134,151
Heparan-sulphate proteoglycans	Matrix growth factor storage (heparanase substrate)	Heparin fragments and analogues; sulphotransferase inhibitors; xylosides; antisense RNA to perlecan	Breast, colon, hepatic, lymphoma, melanoma	68,69,72
Chondroitin-sulphate proteoglycans	Modulate tumour–matrix attachment	Xylosides	Melanoma, glioma, lung	61–63
Hyaluronan	Coordinate tumour growth signalling with cytoskeletal events during migration	Target tumour hyaluronan receptors (for example, gene silencing of CD44)	Breast	28
Tumour metastasis				
O-glycans	Facilitate tumour adhesion during haematogenous metastasis (SLe^X, SLe^A and other selectin ligands);	Disaccharide primers of glycosylation (reduce tumour SLeX); competition by intravenous heparin	Colon	101,112,118
N-linked and *O*-linked glycans	Promote tumour aggregation (galectin-3 binding)	Galectin-3 inhibitors (β-galactosides)	Melanoma	150
Glycosphingolipids	Tumour adhesion (sulphated selectin ligands)	Disaccharide primers; competition with heparin	Colon	101,118
Tumour angiogenesis				
N-glycans	Promote migration of endothelia	Alkaloid inhibitors of *N*-linked glycosylation	Prostate	152
Heparan-sulphate proteoglycans	Co-receptor for growth factors; matrix growth factor storage; co-receptor for matrix proteins	Heparin fragments and analogues; sulphotransferase inhibitors; xylosides; antisense RNA to perlecan	Colon, renal, melanoma, breast	9,71
Tumour immunity				
Glycosphingolipids	Immune 'silencing' (ganglioside shedding)	Ganglioside vaccines	Melanoma, neuroblastoma, breast	35,36,134

O-GlcNAc, *O*-linked N-acetylglucosamine; SLe, sialyl Lewis.

© 2005 Nature Publishing Group

and differentiation factors such as bone morphogenic proteins[27]. Therefore, HSPGs might have important roles in maintaining epithelial differentiation and suppressing progression to malignancy. Similarly, heterozygous mutations in *EXT1* and *EXT2*, which encode the heparan-sulphate copolymerase, result in benign osteochondromas in hereditary multiple exostoses, a paediatric bone disorder in which patients are predisposed to developing malignant chondrosarcomas. The signalling pathway that underlies this disorder is unknown, but might involve Indian Hedgehog (IHH) or FGF18 (known to bind heparan sulphate), which coordinate cartilage growth and bone deposition. Whether HSPGs act as tumour promoters or suppressors probably depends on the array of growth factors and the cell-type-specific regulation of heparan-sulphate formation.

Growth-regulatory effects of plasma membrane-, cytosolic- and nuclear-assembled glycans. The glycans of glycoproteins, proteoglycans and glycosphingolipids are assembled as they pass through the endoplasmic reticulum and Golgi, but a few examples exist in which glycosylation occurs in the plasma membrane, the cytoplasm or the nucleus. Hyaluronan, a large, anionic glycosaminoglycan, is polymerized at the plasma membrane and secreted into the ECM. Tumour matrices are especially rich in hyaluronan. Interactions between hyaluronan and its main receptor CD44 (a transmembrane glycoprotein) as well as CD168, activate signal-transduction pathways that promote cytoskeletal changes involved in cell motility and growth[28]. Studies of tumours transfected with an anti-hyaluronan synthase cDNA have shown that hyaluronan has an important role in tumour proliferation and survival[29]. Receptor activation by tumour hyaluronan glycans promotes signalling and proliferation through the mitogen-activated protein kinase pathway and the phosphatidylinositol 3-kinase (PI3K)–AKT survival pathway. Interestingly, hyaluronan oligomers compete with endogenous polymeric hyaluronan and block signalling responses in breast tumour cells[30].

Many soluble oncogene products and tumour-suppressor proteins contain *O*-GlcNAc (FIG. 1). The addition of *O*-GlcNAc to specific serine residues on the MYC proto-oncogene protein by *O*-GlcNAc transferase promotes mitogenesis in a manner similar to that promoted by phosphorylation of the same amino acids by protein kinases[31]. In another example, *O*-GlcNAc modification of the tumour-suppressor protein p53 appears to mask or block its ability to bind to crucial regions of DNA, and this also promotes growth[31]. These complementary effects of modification by *O*-GlcNAc on major tumour cell-cycle effector proteins make tumour *O*-GlcNAc transferase an appealing target for future cancer therapy.

Therapeutic targeting of glycans that affect tumour proliferation. Given the broad spectrum of glycans that affect tumour proliferation, a range of glycan-specific targeting approaches might be considered for novel drug development. MUC4 represents a possible future target for cancer immunotherapy using anti-mucin monoclonal antibodies or mucin peptide-based vaccines. Other tumour-associated mucins are now being targeted in clinical trials. MUC16 (which carries the CA125 tumour antigen) is currently a target for antibody-based therapy (in Phase II testing). Peptide-based vaccine therapy for targeting MUC1 is in Phase III testing for ovarian and breast carcinomas[12,32,33]. In addition, small monosaccaride and disaccharide decoys — glycan substrates designed to divert (and thereby alter) endogenous glycan synthesis — might also be useful for blocking tumour growth through their ability to alter *O*-glycans expressed on tumour mucins (BOX 1). The use of these mucin-targeting approaches might be limited because secreted- and membrane-bound mucins have important roles in the homeostasis of normal epithelial barriers[12]. Nevertheless, safety has been demonstrated in Phase I studies of MUC1 peptide-based vaccine[34]. Tumour gangliosides are also proving to be appealing targets for tumour-growth inhibition through vaccine approaches[35,36].

Interference with the co-receptor activity of HSPGs on tumour cells represents a novel therapeutic strategy for altering tumour proliferation. This might be achieved, for example, through the inhibition of sulphotransferases that are responsible for sulphating heparan-sulphate chains during biosynthesis, or through the design of competitive blocking agents such as heparan-sulphate mimetics (for example, heparin-based derivatives that have structural similarities to heparan sulphate). One interesting approach is to combine an inhibitor of HSPG synthesis with difluoromethylornithine, an inhibitor of polyamine formation[37,38]. Polyamines are aliphatic cations that are synthesized by all cells during urea metabolism and can be taken up from the extracellular environment. Polyamines function as tumour growth factors through their ability to modulate gene expression and signal transduction[37]. As polyamine uptake depends in part on HSPGs, the combination of the two drugs is sufficient to block tumour growth through inhibition of tumour polyamine uptake and synthesis[38]. Because HSPGs appear to serve important roles in multiple steps during tumour progression, the targeting of HSPGs is discussed in more detail below.

Little is known about possible approaches to target hyaluronan in human cancer, although one promising finding demonstrated the ability of hyaluronan oligomers to inhibit the growth of melanoma in mice[39]. Treatment with either hyaluronan oligomers or the adenoviral administration of hyaluronan-binding protein decoys provide other possible therapeutic avenues[13].

Tumour invasion

During invasion, tumour cells detach from one another and from the ECM and migrate through neighbouring tissue. This requires the remodelling of cell-surface adhesion receptors and ligands, and the secretion of proteolytic enzymes and glycosidases (which catalyse the hydrolysis of glycosidic bonds in

SELECTINS
C-type calcium-dependent lectin expressed by cells in the vasculature and bloodstream. The three known selectins are L-selectin/CD62L (expressed by most leukocytes), E-selectin/CD62E (expressed by cytokine-activated endothelial cells) and P-selectin/CD62P (expressed by activated endothelial cells and platelets). Important ligands for the selectins include glycans containing sialyl Lewis X and sialyl Lewis A.

© 2005 Nature Publishing Group

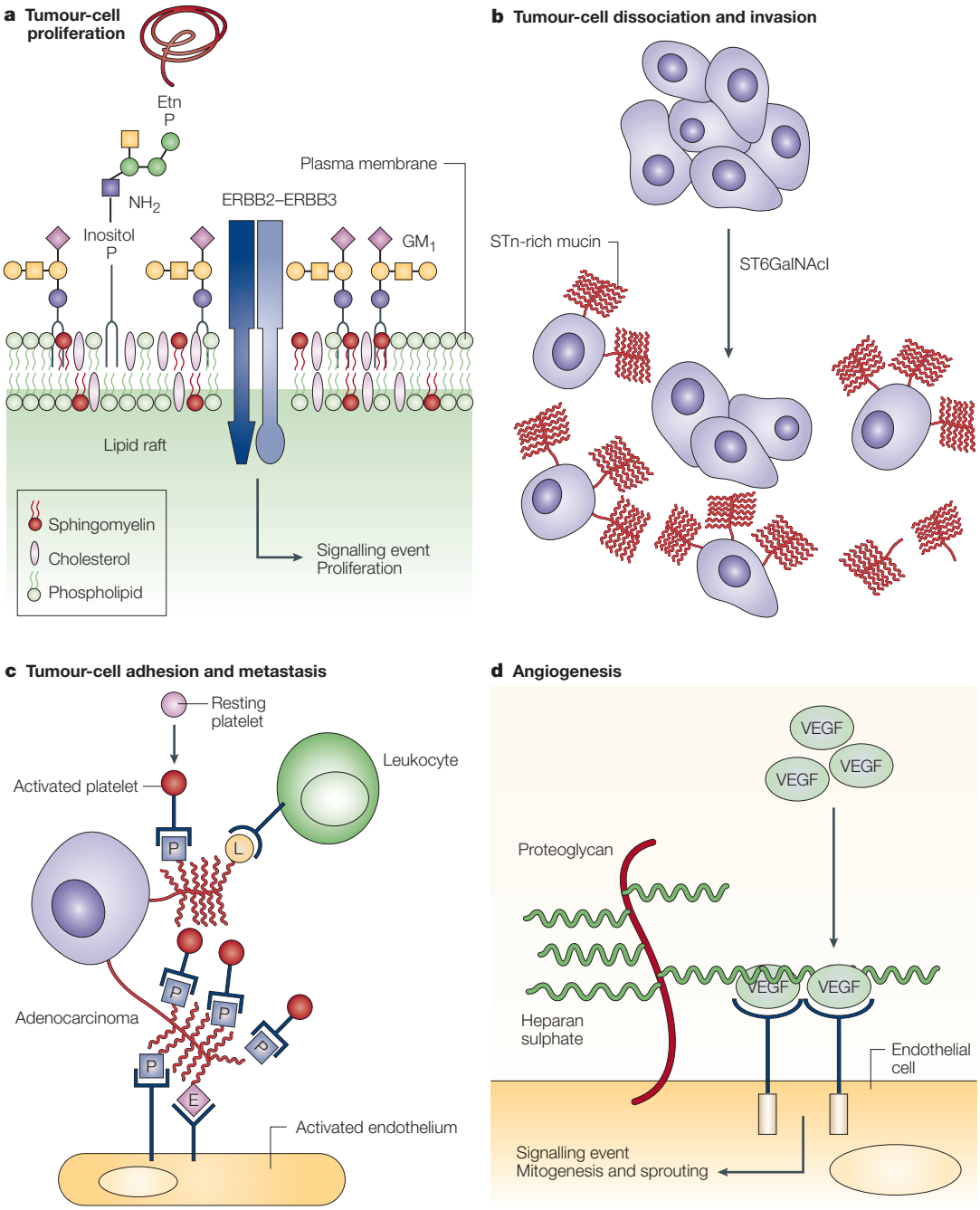


Figure 3 | **Glycans participate in major pathophysiological events during tumour progression.** Tumour proliferation is crucial during early stages of progression and after metastasis, when secondary tumours form at sites distant from the primary tumour. **a** | Various growth-factor receptors are modulated by glycans, for example, through oligomerization of their respective receptor tyrosine kinase receptors in lipid rafts mediated by gangliosides. The figure illustrates the ability of the ganglioside GM_1 to promote ERBB2–ERBB3 receptor heterodimerization. Glycosylphosphatidylinositol-anchored proteins also co-localize in rafts. **b** | Tumour invasion occurs when cells gain the capacity to degrade and migrate through basement membranes and extracellular matrix. Invasion is also associated with the expression of sialylated glycans that promote dissociation of tumour cells. STn is one such class of sialylated glycan overexpressed on tumour cells. **c** | Dissemination of tumour cells through the bloodstream is facilitated by host elements such as platelets and lymphocytes that promote embolization and arrest of tumour at distant endothelial sites. Selectins are important adhesion receptors expressed on activated platelets (P-selectin), leukocytes (L-selectin) and endothelial cells (E-selectin) that bind to specific glycan receptors containing sialyl Lewis X (SLe^X) or SLe^A (expressed in the respective tumour ligands labelled 'P,' 'L', and 'E' in the figure). **d** | Tumour angiogenesis is required for pathological growth of the primary cancer and its metastases. Angiogenesis is the sprouting of blood-vessel endothelial cells in response to pro-angiogenic factors such as vascular endothelial growth factor and fibroblast growth factor (released from the tumour cells and from the host stroma). Heparan sulphate on the endothelial-cell surface facilitates growth-factor binding and activation of endothelial receptor tyrosine kinases.

© 2005 Nature Publishing Group

glycans) to degrade ECM components. Glycans are involved at each of these stages (TABLE 1). Tumours also generate glycoconjugates that appear to serve a repulsive role, physically facilitating the detachment of invasive cells from the primary tumour.

Cell–cell interactions. Malignant cells commonly exhibit increased expression of complex β1,6-branched *N*-linked glycans on their cell surface (FIG. 1), caused by an increase in the expression of the enzyme *N*-acetylglucosaminyltransferase V (GnTV). In the Golgi, this enzyme transfers a GlcNAc residue onto growing *N*-linked glycans so that subsequent glycosylation results in 'multi-antennary' chains[15]. The presence of such complex β1,6-branched *N*-glycans on tumour-cell E-cadherin — an adhesion molecule that normally mediates cell aggregation through homotypic interactions — reduces tumour cell–cell adhesion. Therefore, increased expression of this enzyme promotes cell detachment and invasion[3,8,40,41]. Interestingly, E-cadherin is often downregulated by invasive cancers[42]. Therefore, the coordinated control of E-cadherin and GnTV expression can affect the invasive characteristics of a tumour. The GnTV modification also affects other molecules that are important in modulating tumour adhesion, including the integrin family of adhesion receptors, as discussed below.

Tumour cells tend to produce increased levels of glycoconjugates containing sialic acid, an acidic sugar that imparts a negative charge to the glycan chain (FIG. 1). The formation of polysialic acid also occurs and it is overexpressed on some tumour cells[7,43]. Enhanced sialic acid expression might promote cell detachment from the tumour mass through charge repulsion, which physically inhibits cell–cell apposition. Polysialylation is often associated with the increased invasive potential of tumour cells in both cultured cell lines as well as clinical tumours, and its expression correlates with poor prognosis[7,43,44].

Sialic acid capping of terminal galactose residues on *N*-linked glycans by the enzyme ST6Gal-I (which is upregulated in human breast and colon malignancies) has also been reported to alter tumour cell–cell interactions in a manner that promotes invasion. Transfection of breast cancer cells with this enzyme increases cell migration and reduces cell–cell adhesion, whereas transfection with antisense ST6Gal-I RNA enhances homotypic cell–cell adhesion[45]. Tumour sialic acids could also potentiate invasiveness by receptor-dependent processes or by facilitating interactions between tumour sialic acids and matrix proteins (such as laminin or fibronectin), but additional studies are needed to determine if these processes actually occur.

In tumours, sialic acids are also found on *O*-linked sugars in structures that are rare in normal tissues. A well-known example involves the sialyl Tn antigen (STn), a disaccharide commonly expressed on tumour mucin backbones that consists of the *O*-linked core sugar αGalNAc 'capped' by a sialic acid residue. This glycan is commonly overexpressed in the mucin-rich surfaces of cancer cells (FIG. 1), and its expression potentiates tumour invasiveness. Recent studies have shown that a common defect in many tumours is loss of a chaperone called COSMC, which is required for maturation of the galactosyltransferase that acts on the core GalNAc residue of *O*-linked glycans[46]. Lack of COSMC results in the accumulation of αGalNAc-Ser linkages (Tn antigen), which in turn allows the addition of sialic acid, thereby forming STn antigen. Transfection of breast cancer cells with cDNA that encodes the human sialyltransferase responsible for STn biosynthesis (ST6GalNAc-I) reduces cell–cell interactions and increases the migration potential of transfected cells[47]. STn antigen is also associated with increased metastatic potential, and therefore poor prognosis, in colorectal, gastric and ovarian carcinomas (TABLE 1).

Cell–matrix interactions. Invasion by tumour cells involves the alteration of cell–matrix interactions, which are mediated by adhesion molecules present on the tumour cells that bind to ECM components. Integrins represent a particularly important class of cell-surface adhesion receptors that mediate attachment to important ECM protein ligands, such as fibronectin and laminin. Increased GnTV expression is a hallmark of many malignant cells, and results in increased β1,6 branching on the β_1 subunit of tumour $\alpha_5\beta_1$ integrins, and this disrupts the ability of integrins to cluster on the tumour-cell membrane[48]. Altered integrin clustering, in turn, reduces the formation of tumour-cell focal adhesions, and this increases tumour motility through the ECM as well as invasion across basement membranes. Focal adhesions are supramolecular complexes that link integrin-mediated ECM adhesion to the cytoskeleton as well as initiate signalling pathways that affect cell migration, mechanosensing, and proliferation during cell motility[49].

Whereas the disruption of tumour-cell focal adhesions can promote migration, in some cases the formation of focal adhesions promotes invasion by facilitating tumour-cell spreading on the ECM. During this process, HSPGs on the surface of tumour cells work together with integrins to form focal adhesions[7,23,50–56]. Syndecans are a class of cell-surface-bound HSPGs that have particularly important roles in facilitating these contacts[50–52,57,58]. Syndecan-4 is frequently upregulated in a range of malignancies, including hepatocellular carcinomas and malignant mesotheliomas. It binds to fibronectin and laminin and enhances the function of β1 integrins during cell spreading on matrix[52,58]. Syndecan-1 is overexpressed by a range of human tumours[58], including pancreatic, gastric and breast carcinomas. Its functional coupling with $\alpha_v\beta_3$ integrins on breast carcinoma cells results in $\alpha_v\beta_3$-dependent spreading and migration[59]. Interestingly, in some tumours the downregulation of HSPG on cells can promote anchorage-independent growth[57] and increase invasive potential[58,60]. Therefore, the activity of HSPGs is context dependent. It is also possible that coordinated changes in the expression of specific HSPGs over time might promote sequential

© 2005 Nature Publishing Group

adhesion (which is necessary for tumour-cell spreading) followed by disengagement (which is necessary for motility) during tumour invasion.

Other glycans that might have key roles in tumour–ECM interactions during invasion include chondroitin-sulphate proteoglycans (CSPGs) and hyaluronan (FIG. 1). Chondroitin sulphate is an important constituent of certain tissue matrices (for example, cartilage). Recent studies on brain neoplasms and melanomas show that CSPGs on both the tumour-cell surface and the ECM facilitate tumour invasion by enhancing integrin-mediated cell adhesion, motility and intracellular signalling[13,61–63]. Inhibition of the motility and invasion of melanoma cells across type 1 collagen has been achieved by treating cells with xylosides. Xylosides consist of xylose attached to a hydrophobic aglycone and resemble xylosylated proteoglycan core proteins. Xylosides are able to uncouple chondroitin-sulphate formation from proteoglycan formation by 'decoying' chondroitin-sulphate polymerization away from endogenous CSPG core proteins[63]. The main hyaluronan receptor on tumour cells, CD44, seems to have a prominent role in mediating the binding of tumours to the ECM[64,65]. Interactions between matrix hyaluronan and CD44 also appear to facilitate the tight coordination of tumour-growth signals with cytoskeletal events in tumour migration[28]. Additionally, proteoglycan forms of CD44 that contain chondroitin- and heparan-sulphate chains facilitate the binding of tumour to fibronectin[66,67], thereby affecting the organization of tumour matrix while anchoring tumour cells along a scaffold during invasion.

Matrix degradation and the release of sequestered growth factors. HSPGs secreted into the ECM by the tumour have the capacity to bind and sequester large amounts of important heparin-binding growth factors[68,69] (TABLE 1). This sequestration protects growth factors from denaturation or proteolysis and thereby augments their activity. Moreover, tumour cells that secrete HSPG-digestive enzymes (glycosidases) are able to release and use the glycan-bound factors during matrix invasion[70]. Perlecan, a secreted HSPG that makes up normal basement membranes, has a particularly important role in matrix growth-factor sequestration[71]. Perlecan is overexpressed in several carcinomas, including melanoma, breast and colon malignancies, and transfection of metastatic melanoma cells with perlecan antisense oligonucleotides significantly reduces their invasive potential[72]. Heparanase is an endoglycosidase that partially depolymerizes heparan-suphate chains, and is secreted by invasive normal cells (for example, cytotrophoblasts, which are the specialized epithelial cells in the placenta that have an important role in implantation) and malignant cells (including carcinoma and lymphoma cells). During invasion by tumour cells, heparanases released by migrating tumour cells can liberate sequestered growth factors at an invading tumour front by degrading the heparan-sulphate chains of HSPGs such as perlecan[56,68,69] (TABLE 2). Indeed, the release of heparanase by a range of tumours correlates with metastatic potential[70,73].

Therapeutic targeting of glycans during tumour invasion. From the above discussion, therapeutic targeting of three major glycosylation pathways should be considered as anti-invasion approaches: blockade of tumour *N*-glycosylation (specifically focusing on the inhibition of GnTV); blockade of sialylation pathways in tumours (that is, those that generate polysialic acid or STn); and targeting approaches aimed at HSPGs and CSPGs. The inhibition of tumour hyaluronan might also prove effective.

Inhibition of GnTV promises to be an appealing therapeutic target[74]. However, there are no specific inhibitors of GnTV currently under therapeutic testing. Swainsonine, a competitive inhibitor of *N*-glycan processing in the Golgi (and which partially blocks the action of GnTV), reduced invasion with low toxicity in Phase I human cancer trials[75], and is currently in Phase II testing. The disadvantage of these types of inhibitor is that they affect all *N*-glycan biosynthesis, potentially leading to many side effects. Better knowledge of the crystal structure of GnTV might facilitate the design of specific inhibitors[76]. The isoenzymes ST8SiaII and ST8SiaIV catalyse the synthesis of polysialic acid in mammalian cells. For example, the inhibition of ST8SiaII using synthetic sialic acid precursors has recently been shown[77], but the use of these agents *in vivo* has not been established.

Targeting proteoglycans has also been considered. Administration of clinical heparin inhibits tumour heparanase activity and invasion by tumour cells *in vivo*[55,78], an effect that probably results from the ability of heparin to compete with matrix heparan sulphate as a substrate for tumour heparanase. Furthermore, specific alterations to the length and sulphate-substitution pattern of heparin have been shown to improve its efficacy[55,56,69]. Competitive antagonists of CSPGs might also be considered as possible candidates. Cell-surface CSPGs are overexpressed by certain tumours such as melanoma[79] and breast neoplasms[80]. This observation has been exploited in the laboratory to improve tumour-specific drug delivery (using novel chondroitin-sulphate-binding cationic liposomes loaded with chemotherapeutic agents) and to reduce systemic drug toxicity. Cisplatin (a commonly used chemotherapeutic agent) delivered in this way to tumour-bearing mice resulted in reduced tumour growth, reduced drug toxicity, and improved survival compared with control mice treated with systemic drug[81]. This last example illustrates an alternative approach to the direct targeting of glycans that promote tumour invasion, and demonstrates how one might exploit the tumour's overexpression of certain glycans (for example, proteoglycans or other glycoconjugates) to improve tumour-specific drug targeting and reduce drug toxicity.

© 2005 Nature Publishing Group

Table 2 | **Potentiation of tumour growth by HSPGs and heparin-binding growth factors**

HSPG alterations on tumour cells	Examples of tumours	Mechanism of tumour growth potentiation by HSPG	References
FGF2			
Downregulation of SULF1 (endosulphatase)	Ovarian, breast, hepatocellular, pancreatic, colon, renal	Increase heparan sulphate binding sites for FGF2	25
Increased expression of glypican-1 (a GPI-anchored HSPG)	Pancreatic	Facilitate FGF2 binding to FGF receptors	24,153
HB-EGF			
Downregulation of SULF1 (endosulfatase)	Pancreatic, breast, hepatocellular, colon, ovarian, renal	Increase heparan sulphate binding sites for HB-EGF	25
Increased tumour glypican-1 expression	Breast, pancreatic, ovarian, bladder	Facilitate HB-EGF binding to EGF receptors	154
HGF			
Downregulation of SULF1 (endosulphatase)	Ovarian, breast, hepatocellular, pancreatic, squamous cell	Increase heparan sulphate binding sites for HGF	25
Increased glypican-1 expression	Breast, pancreatic,	Facilitate HGF binding to HGF receptors (MET)	24,153
HGF binding to syndecan-1 (a membrane-bound HSPG)	Multiple myeloma	Facilitate HGF binding and activation of MET	155
VEGF			
Role of specific HSPGs unknown	Breast, ovarian, pancreatic, prostate	Possibly stabilize ligand–receptor ternary complexes; increase matrix growth factor storage	156
Increased expression of perlecan (secreted HSPG)	Melanoma	Possible VEGF reservoir, but role as tumour-cell effector not clear	72
PDGF			
Increased expression of syndecans and perlecan by PDGF	Mesothelioma	PDGF might stimulate increased production of matrix proteoglycans	157
Role of specific HSPGs unknown	Glioblastoma, prostate, gastrointestinal stromal tumours	Unknown	158

FGF2, fibroblast growth factor 2; GPI, glycosylphosphatidylinositol; HB-EGF, heparin-binding epidermal growth factor; HGF, hepatocyte growth factor; HSPG, heparan-sulphate proteoglycan; PDGF, platelet-derived growth factor; SULF1, sulfatase 1; VEGF, vascular endothelial growth factor.

Tumour angiogenesis

Solid tumours and their metastases must acquire a vasculature through the process of tumour angiogenesis in order to achieve diameters of greater than 2 mm[82]. Direct genetic evidence exists that supports a role for HSPGs in tumour angiogenesis[9,71]. The neovasculature that forms during angiogenesis consists of microvascular endothelia, which express 10–15 times the level of HSPG found in macrovascular endothelia[83]. Genetic studies show that endothelial growth and migration are stimulated by several pro-angiogenic factors, including FGF2, VEGF, hepatocyte growth factor (HGF), IL-8, platelet-derived growth factor (PDGF), TGFβ and tumour-necrosis factor-α[7,71], all of which bind heparan sulphate. Furthermore, the endothelial-specific deletion of the important biosynthetic enzyme *N*-deacetylase/*N*-sulphotransferase (NDST1), which is involved in the sulphation of nascent heparan-sulphate chains, diminishes tumour angiogenesis in mouse models (M.M.F. *et al.*, unpublished observations).

Specific HSPGs might have especially crucial roles in tumour angiogenesis. Genetic targeting of the basement-membrane proteoglycan perlecan blocks carcinoma growth and angiogenesis in several *in vivo* models[84,85]. Additionally, the release of heparanase from endothelial cells might promote the cleavage of matrix HSPG, mobilizing pro-angiogenic factors that support the growing tumour neovasculature[56,69,86], making heparanase an attractive target for anti-angiogenesis therapy, as discussed below. Indeed, the importance of mammalian heparanase in vascular remodelling was recently demonstrated by the overexpression of human heparanase in transgenic mice[87].

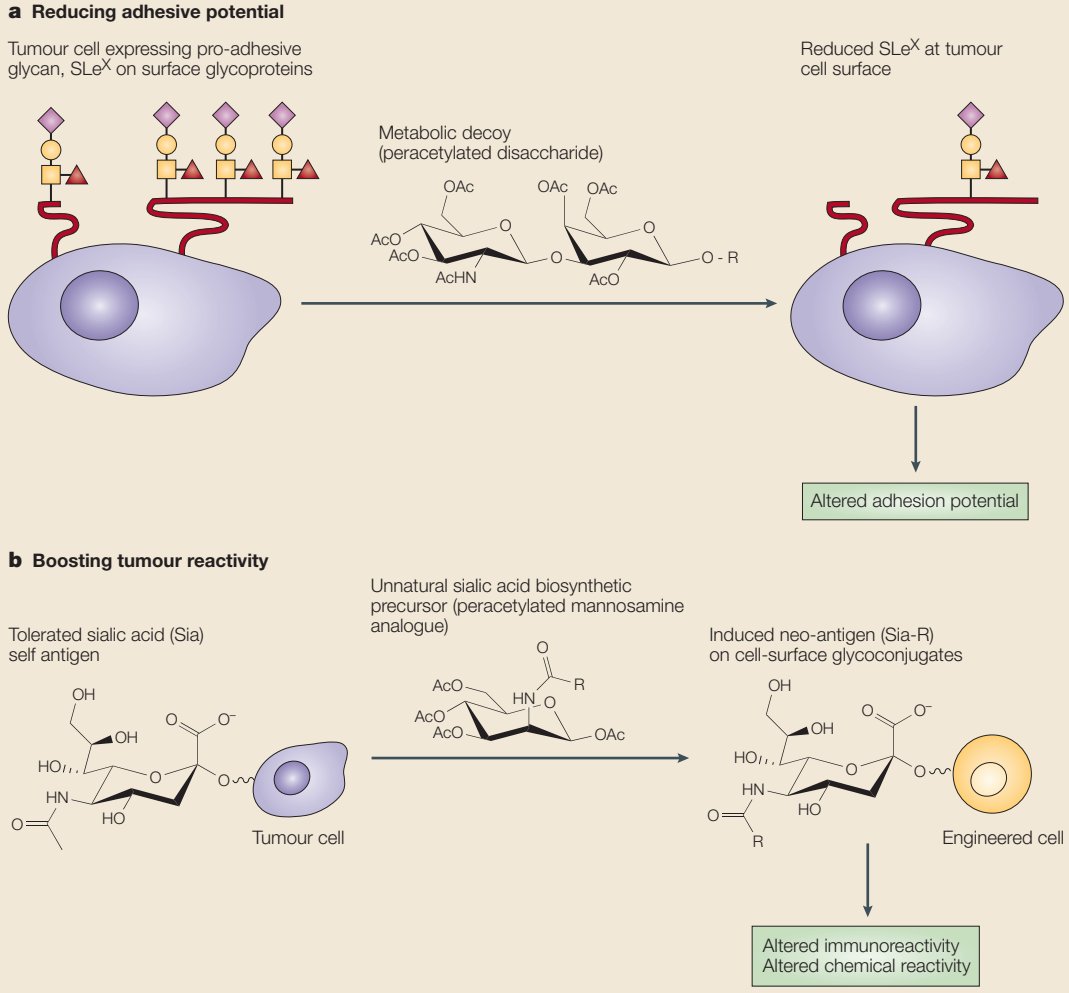
Therapeutic targeting of glycans in tumour angiogenesis. Therapy directed against tumour angiogenesis has the advantage that it might induce less drug resistance than that induced by standard chemotherapy directed against the tumour, because endothelial cells are genetically stable compared with tumour cells[88]. A

© 2005 Nature Publishing Group

Box 1 | Strategies to alter expression of cell-surface glycans on tumour cells

Treatment of tumour cells with peracetylated disaccharides can alter the assembly of cell-surface glycans (see figure part **a**). The figure shows the disaccharide GlcNAcβ1,3Galβ, which is peracetylated and covalently linked to a hydrophobic aglycone (non-carbohydrate 'R' moiety in figure) — these modifications render the disaccharide membrane-permeable. Once inside the tumour cell, the disaccharide is deacetylated and treated as a substrate by one or more glycosyltransferases (which catalyse the transfer of a sugar from a sugar nucleotide donor to a substrate). Assembly of oligosaccharides on the disaccharide 'primer' decoys or diverts glycosylation away from endogenous glycoproteins. This results in decreased expression of specific glycans on the cell surface. In the example, the disaccharide inhibits the expression of sialyl Lewis X (SLe^X) on cell-surface mucins, which blocks tumour dissemination in mice[112]. Other examples of similar decoys include monosaccharide primers such as α-D-*N*-acetylgalactosaminides (for example, GalNAcα-benzyl)[146], which targets *O*-linked glycan biosynthesis, and β-D-xylosides that target heparan-sulphate and chondroitin-sulphate assembly[38,147]. Some analogues of sugars that contain fluorine instead of a specific hydroxyl group directly inhibit glycosylation[148].

Part **b** of the figure depicts another use of glycosides. Tumour cells express sialic acids that are normally tolerated by the immune system, and antitumour immunity can be generated in principle by introducing unnatural sialic acid precursors to tumours, so that their metabolic incorporation leads to the generation of unnatural sialic-acid epitopes. In this example, a mannosamine analogue is fed to tumour cells, where it is converted to a sialic acid derivative. This results in the formation of a cytidine monophosphate-sialic acid derivative that is then used by one or more sialyltransferases to produce a new cell-surface glycoconjugate containing the sialic acid analogue. By adjusting the R-group in the added mannosamine[129,143,144], a range of unnatural chemical structures can be inserted biosynthetically on the cell surface, rendering the cell more immunoreactive or chemically reactive. In this way, it might be possible to boost complement-mediated lysis of the targeted tumour by vaccinating a host with the unnatural sialic acid epitope before administering the analogue. Owing to their high expression of sialic acids, tumour cells might be preferentially targeted by this approach[149].



© 2005 Nature Publishing Group

few groups have achieved glycan-targeted inhibition of angiogenesis through introduction of modified heparin fragments or of sulphated oligosaccharide heparan-sulphate mimetics that specifically inhibit heparanase[89–91]. Both types of agent can also interfere with the ability of heparan sulphate to facilitate endothelial growth-factor signalling by interfering with the formation of growth factor–heparan sulphate–receptor ternary complexes. For example, sulphonic-acid polymers abrogate the formation of the FGF2–HSPG–FGF-receptor complexes and potently inhibit angiogenesis *in vivo*[91,92]. Ongoing research to model the carbohydrate-binding motifs of known glycan-binding growth factors or to probe the binding potential of specific growth factors with libraries of enzymatically treated heparin fragments[93] might yield promising anti-angiogenic compounds. The use of low-molecular-weight heparins should also be explored, as there is some evidence of improved cancer outcomes related to their ability to block angiogenesis[94].

In contrast to heparan sulphate, very little genetic evidence to date implicates other glycan classes in either pathological angiogenesis (which occurs during tumour growth) or physiological angiogenesis. Various cell-surface proteins such as integrins are targets for anti-angiogenic therapy, so one might imagine that the glycosylation of these proteins would affect angiogenesis[95]. Treatment of endothelial cells with deoxymannojirimycin, a plant-derived alkaloid, prevents the synthesis of endothelial hybrid and complex-type *N*-linked oligosaccharides and inhibits the formation of capillary tubes *in vitro*[96], but whether this effect was due to alteration of integrin activity is unknown. Further studies are needed to establish the importance of various glycan classes in tumour endothelial growth and remodelling.

Tumour dissemination through the circulation

Tumour dissemination occurs via the microvasculature, after cells invade the fine capillaries and lymphatics within the tumour. After entry into the vasculature, tumour cells can absorb blood-borne factors, form large aggregates with platelets and leukocytes, and eventually lodge in the small vessels of distant organs. The importance of aggregation of tumour cells with cellular blood components for METASTATIC SEEDING is now well established[97,98].

Over the past two decades, an important role has been established for *O*-glycans in promoting adhesion between tumour cells, platelets and leukocytes by way of receptors known as selectins. Selectins normally mediate adhesion between platelets (which express P-selectin), leukocytes (L-selectin) or endothelium (E- and P-selectins) that bear the glycosylated ligands. The ligands typically consist of clustered arrangements of oligosaccharides that bear sialic acid, fucose and sulphate, presented at the 'tips' of predominantly *O*-linked glycans[99]. One class of carbohydrate ligand consists of the LEWIS TYPE BLOOD GROUP ANTIGENS, sialyl Lewis X (SLe^X) and sialyl Lewis A (SLe^A). The presence of such ligands on blood cells (such as monocytes or neutrophils) or on certain vascular endothelial cells (for example, in lymph nodes) promotes the transmigration of leukocytes to infected and/or inflammatory sites and platelets to sites of vascular damage. Unlike their normal cell counterparts, tumour cells frequently overexpress SLe^X (for example, on lung and colon adenocarcinomas) or SLe^A (for example, on colon, gastric and pancreatic carcinomas) on glycoproteins or glycosphingolipids on the surface of tumour cells[3,100–102]. The expression of these ligands is inversely correlated with patient survival[103–105].

In contrast to the important role of *O*-glycans and glycosphingolipids during the circulatory phase of metastasis, there is little evidence that other glycans potentiate this process. One exception might be endothelial HSPGs, which have the ability to bind several chemokines and facilitate the establishment of chemokine gradients across vascular endothelial layers[159]. The latter appear to potentiate extravasation and metastasis of a range of human tumour cells that commonly overexpress chemokine receptors such as CXCR4 or CXCR7 (REFS 106,107). Furthermore, other lectins such as siglecs (that might modulate binding of innate immune cells to sialic-acid-containing tumour glycans) and galectins (which bind to galactose-containing glycans) might also have important roles in effecting circulatory tumour dissemination[108–110].

Targeting glycans in circulatory tumour dissemination. The mechanism by which tumour-cell aggregates facilitate metastasis has not been established. The coating of tumour cells by platelets and lymphocytes might serve to stabilize tumour emboli composed of activated platelets and leukocytes. The cellular cloak of platelets and leukocytes might facilitate the survival (including protection from innate immunity), microvascular arrest and eventual growth of metastases in the microvasculature of distant organs[100,101,111–113]. Agents that can interfere with selectin–carbohydrate interactions have been considered for the treatment of metastatic disease. These interactions can be targeted through the administration of neutralizing anti-selectin antibodies or small-molecule mimetics of the SLe^X or SLe^A selectin ligands[114–117], but the applicability of such experimental competitive therapies to clinical tumour biology and metastasis remains challenging. Some novel approaches of this type involve the competition by heparin[118] or synthetic negatively charged polymers[119], and the alteration of the tumour ligands themselves through metabolic approaches[112,120,121] (BOX 1). Selectins are also expressed in lymphatic vessels, and might be involved in lymphatic spread of carcinoma[122,123]. Therefore, targeting the glycan ligands for the selectins might serve to limit tumour metastasis through both blood and lymphatic routes. Obviously, these strategies might be limited by undesirable effects on normal processes mediated by the selectin adhesion system (for example, normal leukocyte and platelet functions). However, the dire consequences of metastatic disease warrants further development of this class of therapeutic agents, which might work best in combination with conventional chemotherapy.

METASTATIC SEEDING
The colonization of an organ or tissue by metastatic tumour cells.

LEWIS TYPE BLOOD GROUP ANTIGENS
A structurally similar set of fucose-containing (α1-3-fucosylated) oligosaccharides found on normal epithelia and blood cells, a few of which (for example, the sialyl Lewis X or sialyl Lewis Y antigens) are overexpressed on the surface of certain epithelial tumour cells.

© 2005 Nature Publishing Group

Glycans in tumour immunity

Relatively little is known about the mechanisms by which tumour glycans affect or alter host immunity. Glycosphingolipids might have an important role in certain tumours. Some classes of ganglioside produced by certain tumours can lead to immune silencing though mechanisms discussed below. It is also possible that certain glycan patterns, such as *O*-GlcNAc-modified peptides presented on the tumour surface[124], could stimulate cytotoxic T-cell-mediated responses by the host. Regardless of the mechanisms by which tumour glycosylation influences host immunity, our increasing knowledge of tumour glycan expression means that it is now possible to exploit tumour glycans as a means to augment antitumour immunity.

Augmenting immunity against tumour mucin glycans. A tumour's ability to generate or overexpress unique mucins is now being harnessed in an attempt to improve or augment the immune system's ability to recognize and destroy tumours. Methods to destroy tumour cells using monoclonal antibodies or vaccines that target the tumour mucins MUC1 or MUC16, or the mucin-associated *O*-linked glycan STn, have been introduced as strategies to inhibit tumour proliferation or invasion. Indeed, carcinoma mucins aberrantly glycosylated with either Tn (αGalNAc) or STn often decorate the tumour surface, creating clustered sites for antibody attachment, thereby improving their activity as tumour immunogens. Because glycans are weak immunogens, vaccines of this type are typically prepared by conjugating the glycan to a carrier protein (for example, keyhole limpet haemocyanin (KLH)), and then injecting the compound into the patient together with an adjuvant that boosts T-cell responses. Vaccines that contain these epitopes are currently being used in breast cancer patients to augment antitumour immune responses, and a positive survival effect has been noted in patients treated with the STn–KLH conjugate 'Theratope' (currently in Phase III clinical trials[125]). Ongoing clinical trials are examining the use of this agent to treat ovarian and colorectal cancer patients[126,127]. In addition, peptide mimetics of tumour carbohydrates, such as SLe^X, SLe^A or SL^Y, have also been shown to stimulate tumour immunity, although these studies have only been carried out in animal models[128].

In another promising strategy, antitumour immunity has been generated by making unique alterations to tumour-cell-surface sialic acids (which are normally tolerated by the immune system). This might be accomplished by introducing unnatural sialic acid precursors to tumours (BOX 1), whereby metabolic incorporation leads to the generation of unnatural sialic acid epitopes, forming the basis for the induction of novel antitumour immune responses[129].

Augmenting immunity against tumour glycosphingolipids. Some mammary carcinomas, neuroblastomas, sarcomas, melanomas, small-cell lung carcinomas and lymphomas express very high levels of immunogenic gangliosides, and often shed them into the bloodstream[3,8]. Paradoxically, this can lead to immune silencing, which probably involves both the inhibition of co-stimulatory molecule synthesis as well as the arrest of dendritic-cell maturation, resulting in the inability of dendritic cells to generate effective antitumour T-cell immune responses[130,131]. This observation indicates that inhibitors of the ganglioside assembly process might prove effective in these types of tumour[132,133].

Although glycosphingolipids are relatively poor immunogens, certain glycosphingolipids might be manipulated to generate both passive immunity (by the infusion of antibodies to glycans) and active immunity (by eliciting a host response to a glycan) against tumours. Immunization with purified G_{D2} or G_{M2} gangliosides (FIG. 1) has been carried out, initially with some success in animal models and preliminary human melanoma trials. This approach is showing promise in several current clinical trials against melanoma (using G_{D2}–KLH[134]), neuroblastoma (using anti-G_{D2} small immunoproteins[35]), breast (using $NeuGcG_{M3}$ proteoliposomes[36] that contain the *N*-glycolyl form of sialic acid) and prostate carcinomas (using a conjugate between KLH and Globo-H hexasaccharide[135], a glycan expressed on human prostate and breast cancer glycolipids). Finally, liposomal drug-delivery techniques can take advantage of ganglioside overexpression by certain tumours. For instance, *MYB* antisense oligodeoxynucleotides (for inhibiting the synthesis of MYB) have been delivered to human neuroblastomas by encapsulation in cationic liposomes that have been covalently coupled to monoclonal antibodies against the G_{D2} ganglioside[136].

Measurement of serum tumour glycans

Serum measurement of certain glycans on the surface of tumour cells is currently used to facilitate diagnosis, track tumour recurrence or tumour burden or provide a surrogate measure for therapeutic response. These glycans might be regarded as part of a larger array of 'metastatic codes' that a tumour's glycan profile (or 'glycotype') might represent. For example, the serological markers CA125, CA19-9 and CA15-3 are mucin glycoconjugates that are commonly overexpressed by ovarian, pancreatic and breast adenocarcinomas, respectively, and their serum levels correlate with tumour burden and prognosis[137–139]. In ovarian cancer, the tumour antigen CA125 is associated with a large mucin-like glycoprotein called MUC16 that interacts with galactose-binding lectins (galectins) that are secreted into the tumour matrix. One of the ways in which galectins might regulate tumour progression is through their ability to facilitate cell adhesion and promote the binding of tumour cells to laminin and fibronectin[110]. Their overexpression in ovarian cancer might facilitate tumour–matrix interactions during invasion of ovarian cancer cells[140]. CA19-9 is the epitope that interacts with SLe^A on pancreatic carcinoma mucins[141], and its expression facilitates

© 2005 Nature Publishing Group

Table 3 | **Current clinical targets of glycans in cancer**

Therapeutic agent	Mechanism of action	Clinical status	References
Targeting* N-*glycans			
GD0039 (Swainsonine)	Blocks Golgi α-mannosidase	Phase II (renal cancer)	11
Targeting O-glycans			
Theratope	Conjugated vaccine targets the STn mucin epitope	Phase III (breast cancer)	125
Disaccharide primers (peracetylated GlcNAcβ1,3Gal-naphthalen-emethanol)	Alter SLe^X (selectin ligand facilitating haematogenous metastasis)	Preclinical	112
Heparin	Inhibits P-selectin and L-selectin binding to SLe^X ligand	On the market	118
Targeting heparan-sulphate proteoglycans			
Heparin	Inhibits heparanase and interactions between growth factor and heparan sulphate	On the market	78
PI88	Inhibits FGF release via heparanase inhibition	Phase I and II (melanoma) Phase II (multiple myeloma)	11
Targeting gangliosides			
GMK vaccine	Conjugated G_{M2} vaccine	Phase III (melanoma)	11
NeuGc G_{M3}/VSSP	NeuGcG_{M3} proteoliposomes	Phase I (breast)	36
Anti-G_{D2} SIP	Anti-G_{D2} small immunoproteins	Preclinical	35
G_{D2} lactone–KLH	Conjugated G_{D2} vaccine	Phase I (melanoma)	134
Targeting galectin-binding glycans			
GCS-100 (citrus pectin derivative)	Blocks galectin-3 binding; inhibits tumour apoptosis; facilitates tumour aggregation	Phase II (pancreatic and colon cancers)	150

selectin-mediated adhesion during haematogenous metastasis. In breast cancer, the tumour antigen CA15-3 is expressed on MUC1, which is aberrantly expressed in more than 90% of breast carcinomas and appears to promote invasion[142]. Therefore, the glycans CA125, CA19-9 and CA15-3 are examples of molecules that not only serve as tumour markers for diagnosis, but also appear to serve important pathophysiological roles in cancer progression. For now, although these and other glycan diagnostic markers are used clinically as sensitive markers for recurrence of disease following initial treatment, they might also be used to facilitate the timing of glycan-based therapies in future cancer treatment programmes.

Implications and future directions

A few important patterns emerge from the above discussion. First, a given glycan might act at different stages of tumour progression, so targeting that glycan might have broad effects (FIG. 3). Second, at any given stage of progression, a specific glycan-targeting strategy might alter several glycan-dependent interactions. For example, altering selectin interactions would affect platelet, lymphocyte and endothelial adhesion. Third, some therapeutic strategies can target more than one class of glycan–protein interaction. For example, heparin will block selectin–SLe^X interactions and also block invasion and angiogenesis by inhibiting heparanases and growth-factor interactions with their receptors. Alternatively, metabolic decoys might alter tumour-cell interactions that are mediated by selectins and affect the immunogenicity of carcinoma mucins (BOX 1). Altering *N*-glycosylation of tumour glycoproteins might confer additional biological effects by affecting the folding of several crucial membrane proteins[145]. Finally, one should also consider the potential synergistic effects of altering tumour glycans in combination with existing radiotherapeutic and chemotherapeutic therapies or anti-angiogenic therapies. An added advantage of this approach is that it might also overcome the problems of tumour heterogeneity and drug resistance, which are concerns that are inherent in any specific targeting of tumour cells.

Ultimately, the choice and timing of any future glycan-based therapy against cancer should be guided by both serological assays for glycan markers as well as novel biopsy information. Refinement of technologies to analyse clinical biopsy material (yielding a glycotype

© 2005 Nature Publishing Group

for the tumour) in addition to serum glycans might assist with the rational choice and/or early application of glycan-based immunomodulators. Glycan analysis of serum or biopsy material could also facilitate the preoperative administration of tumour-glycan inhibitors to reduce the risk of operation-associated dissemination of microscopic tumour deposits. The recent application of microarray technology might provide insights into the array of enzymes and glycoproteins associated with the grade and stage of a given tumour. This, in turn, might indicate appropriate tumour and patient-specific panels of inhibitors to test. Valuable pharmacological approaches might then result from profiling drug design according to genetically validated targets.

As we continue to uncover clues about pathogenic mechanisms in tumour progression that glycans facilitate, or in some cases, govern, the integration of glycan-targeted therapy with existing cancer treatment protocols might have significant impact on disease outcomes. Progress so far on targeting complex carbohydrates in cancer (summarized in TABLE 3) might represent the 'tip of an iceberg' of therapeutic potential that awaits future discovery.

1. Wells, L., Vosseller, K. & Hart, G. W. Glycosylation of nucleocytoplasmic proteins: signal transduction and *O*-GlcNAc. *Science* **291**, 2376–2378 (2001).
2. Marth, J. Glycosylation changes in ontogeny and cell activation. in *Essentials of Glycobiology* (eds Varki, A. *et al.*) 515–536 (Cold Spring Harbor Laboratory Press, New York, 1999).
3. Varki, A. in *Essentials of Glycobiology* (eds Varki, A. *et al.*) 537–549 (Cold Spring Harbor Laboratory Press, New York, 1999).
4. Feizi, T. Carbohydrate antigens in human cancer. *Cancer Surv.* **4**, 245–269 (1985).
5. Raedler, A. & Schreiber, S. Analysis of differentiation and transformation of cells by lectins. *Crit. Rev. Clin. Lab. Sci.* **26**, 153–193 (1988).
6. Weis, W. I. & Drickamer, K. Structural basis of lectin-carbohydrate recognition. *Annu. Rev. Biochem.* **65**, 441–473 (1996).
Describes the structural basis for sugar recognition and adhesion by lectins expressed in bacteria, animals and plants.
7. Bogenrieder, T. & Herlyn, M. Axis of evil: molecular mechanisms of cancer metastasis. *Oncogene* **22**, 6524–6536 (2003).
8. Hakomori, S. Tumor malignancy defined by aberrant glycosylation and sphingo(glyco)lipid metabolism. *Cancer Res.* **56**, 5309–5318 (1996).
9. Iozzo, R. V. & San Antonio, J. D. Heparan sulfate proteoglycans: heavy hitters in the angiogenesis arena. *J. Clin. Invest.* **108**, 349–355 (2001).
10. Liu, D., Shriver, Z., Qi, Y., Venkataraman, G. & Sasisekharan, R. Dynamic regulation of tumor growth and metastasis by heparan sulfate glycosaminoglycans. *Semin. Thromb. Hemost.* **28**, 67–78 (2002).
11. Maeder, T. Sweet medicines. *Sci. Am.* **287**, 40–47 (2002).
12. Hollingsworth, M. A. & Swanson, B. J. Mucins in cancer: protection and control of the cell surface. *Nature Rev. Cancer* **4**, 45–60 (2004).
13. Toole, B. P. Hyaluronan: from extracellular glue to pericellular cue. *Nature Rev. Cancer* **4**, 528–539 (2004).
14. Felsher, D. W. Reversibility of oncogene-induced cancer. *Curr. Opin. Genet. Dev.* **14**, 37–42 (2004).
15. Marth, J. in *Essentials of Glycobiology* (eds Varki, A. *et al.*) 85–100 (Cold Spring Harbor Laboratory Press, New York, 1999).
16. Girnita, L. *et al.* Inhibition of *N*-linked glycosylation down-regulates insulin-like growth factor-1 receptor at the cell surface and kills Ewing's sarcoma cells: therapeutic implications. *Anticancer Drug Des.* **15**, 67–72 (2000).
17. Bharathan, S., Moriarty, J., Moody, C. E. & Sherblom, A. P. Effect of tunicamycin on sialomucin and natural killer susceptibility of rat mammary tumor ascites cells. *Cancer Res.* **50**, 5250–5256 (1990).
18. Komatsu, M., Jepson, S., Arango, M. E., Carothers Carraway, C. A. & Carraway, K. L. Muc4/sialomucin complex, an intramembrane modulator of ErbB2/HER2/Neu, potentiates primary tumor growth and suppresses apoptosis in a xenotransplanted tumor. *Oncogene* **20**, 461–470 (2001).
Demonstrates how genetic upregulation of MUC4 in tumour A375 melanoma cells inhibits apoptosis of tumour cells and promotes tumour growth *in vivo*. MUC4 modulates phosphorylation of the receptor tyrosine kinase in the presence and absence of the major EGF receptor ligand, heregulin.
19. Hakomori, S. Bifunctional role of glycosphingolipids. Modulators for transmembrane signaling and mediators for cellular interactions. *J. Biol. Chem.* **265**, 18713–18716 (1990).
20. Nagy, P. *et al.* Lipid rafts and the local density of ErbB proteins influence the biological role of homo- and heteroassociations of ErbB2. *J. Cell Sci.* **115**, 4251–4262 (2002).
21. Esko, J. D. in *Essentials of Glycobiology* (eds Varki, A. *et al.*) 145–159 (Cold Spring Harbor Laboratory Press, New York, 1999).
22. Esko, J. D. & Lindahl, U. Molecular diversity of heparan sulfate. *J. Clin. Invest.* **108**, 169–173 (2001).
23. Bernfield, M. *et al.* Functions of cell surface heparan sulfate proteoglycans. *Annu. Rev. Biochem.* **68**, 729–777 (1999).
24. Kleeff, J. *et al.* The cell-surface heparan sulfate proteoglycan glypican-1 regulates growth factor action in pancreatic carcinoma cells and is overexpressed in human pancreatic cancer. *J. Clin. Invest.* **102**, 1662–1673 (1998).
25. Lai, J. *et al.* Loss of HSulf-1 up-regulates heparin-binding growth factor signaling in cancer. *J. Biol. Chem.* **278**, 23107–23117 (2003).
Demonstration of the importance of HSPGs in tumour proliferation. The cell-surface-associated enzyme SULF1 diminishes HSPG sulphation. Downregulation of SULF1 by tumours increases growth-factor signalling and resistance to apoptosis, representing a novel mechanism by which cancer cells can enhance growth.
26. DeBaun, M. R., Ess, J. & Saunders, S. Simpson Golabi Behmel syndrome: progress toward understanding the molecular basis for overgrowth, malformation, and cancer predisposition. *Mol. Genet. Metab.* **72**, 279–286 (2001).
27. Filmus, J. Glypicans in growth control and cancer. *Glycobiology* **11**, 19R–23R (2001).
28. Turley, E. A., Noble, P. W. & Bourguignon, L. Y. Signaling properties of hyaluronan receptors. *J. Biol. Chem.* **277**, 4589–4592 (2002).
29. Simpson, M. A., Wilson, C. M. & McCarthy, J. B. Inhibition of prostate tumor cell hyaluronan synthesis impairs subcutaneous growth and vascularization in immunocompromised mice. *Am. J. Pathol.* **161**, 849–857 (2002).
30. Misra, S., Ghatak, S., Zoltan-Jones, A. & Toole, B. P. Regulation of multidrug resistance in cancer cells by hyaluronan. *J. Biol. Chem.* **278**, 25285–25288 (2003).
31. Chou, T. Y. & Hart, G. W. *O*-linked *N*-acetylglucosamine and cancer: messages from the glycosylation of c-Myc. *Adv. Exp. Med. Biol.* **491**, 413–418 (2001).
Discusses the importance of modification by *O*-GlcNAc of key cellular oncogenes or tumour-suppressor proteins. *O*-GlcNAc transferase glycosylates tumour-suppressor proteins at amino-acid residues that either promote oncogene activity or inhibit tumour-suppressor functions, highlighting the importance of *O*-GlcNAc in tumorigenesis and tumour proliferation.
32. Noujaim, A. A., Schultes, B. C., Baum, R. P. & Madiyalakan, R. Induction of CA125-specific B and T cell responses in patients injected with MAb-B43.13 — evidence for antibody-mediated antigen-processing and presentation of CA125 *in vivo*. *Cancer Biother. Radiopharm.* **16**, 187–203 (2001).
33. Musselli, C. *et al.* Reevaluation of the cellular immune response in breast cancer patients vaccinated with MUC1. *Int. J. Cancer* **97**, 660–667 (2002).
34. Ramanathan, R. K. *et al.* Phase I study of a MUC1 vaccine composed of different doses of MUC1 peptide with SB-AS2 adjuvant in resected and locally advanced pancreatic cancer. *Cancer Immunol. Immunother.* **54**, 254–264 (2005).
35. Bestagno, M., Occhino, M., Corrias, M. V., Burrone, O. & Pistoia, V. Recombinant antibodies in the immunotherapy of neuroblastoma: perspectives of new developments. *Cancer Lett.* **197**, 193–198 (2003).
36. Carr, A. *et al.* Immunotherapy of advanced breast cancer with a heterophilic ganglioside (NeuGcGM3) cancer vaccine. *J. Clin. Oncol.* **21**, 1015–1021 (2003).
37. Thomas, T. & Thomas, T. J. Polyamine metabolism and cancer. *J. Cell Mol. Med.* **7**, 113–126 (2003).
38. Belting, M. *et al.* Tumor attenuation by combined heparan sulfate and polyamine depletion. *Proc. Natl Acad. Sci. USA* **99**, 371–376 (2002).
39. Zeng, C., Toole, B. P., Kinney, S. D., Kuo, J. W. & Stamenkovic, I. Inhibition of tumor growth *in vivo* by hyaluronan oligomers. *Int. J. Cancer* **77**, 396–401 (1998).
Disruption of the interaction between host tissue stromal hyaluronan and the hyaluronan receptor CD44 on tumour cells can be achieved by treatment with hyaluronan oligomers. This study illustrates how infusion with such oligomers can block growth of experimental melanomas *in vivo*.
40. Dennis, J. W., Pawling, J., Cheung, P., Partridge, E. & Demetriou, M. UDP-*N*-acetylglucosamine:α-6-D-mannoside β1, 6 *N*-acetylglucosaminyltransferase V (*Mgat5*) deficient mice. *Biochim. Biophys. Acta.* **1573**, 414–422 (2002).
41. Yoshimura, M., Ihara, Y., Matsuzawa, Y. & Taniguchi, N. Aberrant glycosylation of E-cadherin enhances cell–cell binding to suppress metastasis. *J. Biol. Chem.* **271**, 13811–13815 (1996).
42. Hirohashi, S. & Kanai, Y. Cell adhesion system and human cancer morphogenesis. *Cancer Sci.* **94**, 575–581 (2003).
43. Seidenfaden, R., Krauter, A., Schertzinger, F., Gerardy-Schahn, R. & Hildebrandt, H. Polysialic acid directs tumor cell growth by controlling heterophilic neural cell adhesion molecule interactions. *Mol. Cell. Biol.* **23**, 5908–5918 (2003).
This study examines the role of polysialic acid on neural cell adhesion molecule (NCAM) in neuroblastoma cells, and demonstrates the ability of polysialic acid to control tumour-cell growth and differentiation by interfering with NCAM signalling at cell–cell contacts.
44. Angata, K. & Fukuda, M. Polysialyltransferases: major players in polysialic acid synthesis on the neural cell adhesion molecule. *Biochimie* **85**, 195–206 (2003).
45. Lin, S., Kemmner, W., Grigull, S. & Schlag, P. M. Cell surface α 2, 6 sialylation affects adhesion of breast carcinoma cells. *Exp. Cell Res.* **276**, 101–110 (2002).
46. Ju, T. & Cummings, R. D. A unique molecular chaperone Cosmc required for activity of the mammalian core 1β 3-galactosyltransferase. *Proc. Natl Acad. Sci. USA* **99**, 16613–16618 (2002).
47. Julien, S. *et al.* Expression of sialyl-Tn antigen in breast cancer cells transfected with the human CMP-Neu5Ac: GalNAc α2, 6-sialyltransferase (ST6GalNac I) cDNA. *Glycoconj. J.* **18**, 883–893 (2001).
48. Guo, H. B., Lee, I., Kamar, M., Akiyama, S. K. & Pierce, M. Aberrant *N*-glycosylation of β1 integrin causes reduced α5β1 integrin clustering and stimulates cell migration. *Cancer Res.* **62**, 6837–6845 (2002).
49. Wozniak, M. A., Modzelewska, K., Kwong, L. & Keely, P. J. Focal adhesion regulation of cell behavior. *Biochim. Biophys. Acta* **1692**, 103–119 (2004).
50. Tumova, S., Woods, A. & Couchman, J. R. Heparan sulfate proteoglycans on the cell surface: versatile coordinators of cellular functions. *Int. J. Biochem. Cell Biol.* **32**, 269–288 (2000).

© 2005 **Nature Publishing Group**

51. Woods, A., McCarthy, J. B., Furcht, L. T. & Couchman, J. R. A synthetic peptide from the COOH-terminal heparin-binding domain of fibronectin promotes focal adhesion formation. *Mol. Biol. Cell* **4**, 605–613 (1993).
52. Saoncella, S. *et al.* Syndecan-4 signals cooperatively with integrins in a Rho-dependent manner in the assembly of focal adhesions and actin stress fibers. *Proc. Natl Acad. Sci. USA* **96**, 2805–2810 (1999).
53. Kusano, Y., Yoshitomi, Y., Munesue, S., Okayama, M. & Oguri, K. Cooperation of syndecan-2 and syndecan-4 among cell surface heparan sulfate proteoglycans in the actin cytoskeletal organization of Lewis lung carcinoma cells. *J. Biochem. (Tokyo)* **135**, 129–137 (2004).
54. Culp, L. A. *et al.* Heparan sulfate proteoglycans of Ras-transformed 3T3 or neuroblastoma cells. Differing functions in adhesion on fibronectin. *Ann. NY Acad. Sci.* **556**, 194–216 (1989).
55. Vlodavsky, I. *et al.* Inhibition of tumor metastasis by heparanase inhibiting species of heparin. *Invasion Metastasis* **14**, 290–302 (1994).
56. Vlodavsky, I. & Friedmann, Y. Molecular properties and involvement of heparanase in cancer metastasis and angiogenesis. *J. Clin. Invest.* **108**, 341–347 (2001).
Reviews the many roles of heparanase in the tumour environment, and its importance in tumour progression.
57. Kato, M., Saunders, S., Nguyen, H. & Bernfield, M. Loss of cell surface syndecan-1 causes epithelia to transform into anchorage-independent mesenchyme-like cells. *Mol. Biol. Cell* **6**, 559–576 (1995).
58. Beauvais, D. M. & Rapraeger, A. C. Syndecans in tumor cell adhesion and signaling. *Reprod. Biol. Endocrinol.* **2**, 3 (2004).
59. Beauvais, D. M., Burbach, B. J. & Rapraeger, A. C. The syndecan-1 ectodomain regulates $\alpha_v\beta_3$ integrin activity in human mammary carcinoma cells. *J. Cell Biol.* **167**, 171–181 (2004).
60. Sanderson, R. D. Heparan sulfate proteoglycans in invasion and metastasis. *Semin. Cell Dev. Biol.* **12**, 89–98 (2001).
61. Iida, J., Meijne, A. M., Knutson, J. R., Furcht, L. T. & McCarthy, J. B. Cell surface chondroitin sulfate proteoglycans in tumor cell adhesion, motility and invasion. *Semin. Cancer Biol.* **7**, 155–162 (1996).
62. Nutt, C. L., Zerillo, C. A., Kelly, G. M. & Hockfield, S. Brain enriched hyaluronan binding (BEHAB)/brevican increases aggressiveness of CNS-1 gliomas in Lewis rats. *Cancer Res.* **61**, 7056–7059 (2001).
63. Faassen, A. E. *et al.* A cell surface chondroitin sulfate proteoglycan, immunologically related to CD44, is involved in type I collagen-mediated melanoma cell motility and invasion. *J. Cell Biol.* **116**, 521–531 (1992).
64. Gunthert, U. *et al.* A new variant of glycoprotein CD44 confers metastatic potential to rat carcinoma cells. *Cell* **65**, 13–24 (1991).
65. Aruffo, A., Stamenkovic, I., Melnick, M., Underhill, C. B. & Seed, B. CD44 is the principal cell surface receptor for hyaluronate. *Cell* **61**, 1303–1313 (1990).
66. Jalkanen, S. & Jalkanen, M. Lymphocyte CD44 binds the COOH-terminal heparin-binding domain of fibronectin. *J. Cell Biol.* **116**, 817–825 (1992).
67. Wolff, E. A. *et al.* Generation of artificial proteoglycans containing glycosaminoglycan-modified CD44. Demonstration of the interaction between rantes and chondroitin sulfate. *J. Biol. Chem.* **274**, 2518–2524 (1999).
68. Vlodavsky, I. *et al.* Mammalian heparanase: gene cloning, expression and function in tumor progression and metastasis. *Nature Med.* **5**, 793–802 (1999).
69. Goldshmidt, O. *et al.* Cell surface expression and secretion of heparanase markedly promote tumor angiogenesis and metastasis. *Proc. Natl Acad. Sci. USA* **99**, 10031–10036 (2002).
70. Vlodavsky, I. *et al.* Molecular properties and involvement of heparanase in cancer progression and normal development. *Biochimie* **83**, 831–839 (2001).
71. Jiang, X. & Couchman, J. R. Perlecan and tumor angiogenesis. *J. Histochem. Cytochem.* **51**, 1393–1410 (2003).
72. Adatia, R. *et al.* Suppression of invasive behavior of melanoma cells by stable expression of anti-sense perlecan cDNA. *Ann. Oncol.* **8**, 1257–1261 (1997).
73. Zcharia, E. *et al.* Molecular properties and involvement of heparanase in cancer progression and mammary gland morphogenesis. *J. Mammary Gland Biol. Neoplasia* **6**, 311–322 (2001).
74. Granovsky, M. *et al.* Suppression of tumor growth and metastasis in Mgat5-deficient mice. *Nature Med.* **6**, 306–312 (2000).
Demonstrates the importance of *N*-acetylglucosaminyltransferase V (GnTV) in tumour progression by examining mammary tumour growth and metastasis in mice bearing a deletion in the enzyme.
75. Goss, P. E., Baptiste, J., Fernandes, B., Baker, M. & Dennis, J. W. A phase I study of swainsonine in patients with advanced malignancies. *Cancer Res.* **54**, 1450–1457 (1994).
76. Schachter, H. The joys of HexNAc. The synthesis and function of *N*- and *O*-glycan branches. *Glycoconj. J.* **17**, 465–483 (2000).
77. Horstkorte, R. *et al.* Selective inhibition of polysialyltransferase ST8SiaII by unnatural sialic acids. *Exp. Cell Res.* **298**, 268–274 (2004).
78. Kakkar, A. K. An expanding role for antithrombotic therapy in cancer patients. *Cancer Treat. Rev.* **29** (Suppl. 2), 23–26 (2003).
79. Bumol, T. F. & Reisfeld, R. A. Unique glycoprotein-proteoglycan complex defined by monoclonal antibody on human melanoma cells. *Proc. Natl Acad. Sci. USA* **79**, 1245–1249 (1982).
80. Olsen, E. B., Trier, K., Eldov, K. & Ammitzboll, T. Glycosaminoglycans in human breast cancer. *Acta Obstet. Gynecol. Scand.* **67**, 539–542 (1988).
81. Lee, C. M. *et al.* Novel chondroitin sulfate-binding cationic liposomes loaded with cisplatin efficiently suppress the local growth and liver metastasis of tumor cells *in vivo*. *Cancer Res.* **62**, 4282–4288 (2002).
82. Folkman, J. in *The Molecular Basis of Cancer* (eds Mendelsohn, J., Howley P. M., Israel M. A. & Liotta L. A.) 206–232 (W. B. Saunders, Philadelphia, 1995).
83. Marcum, J. A. & Rosenberg, R. D. Heparinlike molecules with anticoagulant activity are synthesized by cultured endothelial cells. *Biochem. Biophys. Res. Commun.* **126**, 365–372 (1985).
84. Zhou, Z. *et al.* Impaired angiogenesis, delayed wound healing and retarded tumor growth in perlecan heparan sulfate-deficient mice. *Cancer Res.* **64**, 4699–4702 (2004).
85. Sharma, B. *et al.* Antisense targeting of perlecan blocks tumor growth and angiogenesis *in vivo*. *J. Clin. Invest.* **102**, 1599–1608 (1998).
86. Folkman, J. & Shing, Y. Control of angiogenesis by heparin and other sulfated polysaccharides. *Adv. Exp. Med. Biol.* **313**, 355–364 (1992).
87. Zcharia, E. *et al.* Transgenic expression of mammalian heparanase uncovers physiological functions of heparan sulfate in tissue morphogenesis, vascularization, and feeding behavior. *FASEB J.* **18**, 252–263 (2004).
88. Boehm, T., Folkman, J., Browder, T. & O'Reilly, M. S. Antiangiogenic therapy of experimental cancer does not induce acquired drug resistance. *Nature* **390**, 404–407 (1997).
89. Lapierre, F. *et al.* Chemical modifications of heparin that diminish its anticoagulant but preserve its heparanase-inhibitory, angiostatic, anti-tumor and anti-metastatic properties. *Glycobiology* **6**, 355–366 (1996).
90. Parish, C. R., Freeman, C., Brown, K. J., Francis, D. J. & Cowden, W. B. Identification of sulfated oligosaccharide-based inhibitors of tumor growth and metastasis using novel *in vitro* assays for angiogenesis and heparanase activity. *Cancer Res.* **59**, 3433–3441 (1999).
91. Presta, M. *et al.* Heparin derivatives as angiogenesis inhibitors. *Curr. Pharm. Des.* **9**, 553–566 (2003).
92. Liekens, S. *et al.* Modulation of fibroblast growth factor-2 receptor binding, signaling, and mitogenic activity by heparin-mimicking polysulfonated compounds. *Mol. Pharmacol.* **56**, 204–213 (1999).
93. Jemth, P. *et al.* Biosynthetic oligosaccharide libraries for identification of protein-binding heparan sulfate motifs. Exploring the structural diversity by screening for fibroblast growth factor (FGF)1 and FGF2 binding. *J. Biol. Chem.* **277**, 30567–30573 (2002).
94. Pisano, C. *et al.* Undersulfated, low-molecular-weight glycol-split heparin as an antiangiogenic VEGF antagonist. *Glycobiology* **15**, 1C–6C (2005).
95. Hwang, R. & Varner, J. The role of integrins in tumor angiogenesis. *Hematol. Oncol. Clin. North Am.* **18**, 991–1006, vii (2004).
96. Nguyen, M., Folkman, J. & Bischoff, J. 1-Deoxymannojirimycin inhibits capillary tube formation *in vitro*. Analysis of *N*-linked oligosaccharides in bovine capillary endothelial cells. *J. Biol. Chem.* **267**, 26157–26165 (1992).
97. Krause, T. & Turner, G. A. Are selectins involved in metastasis? *Clin. Exp. Metastasis* **17**, 183–192 (1999).
98. Borsig, L. *et al.* Pictures in molecular medicine: three-dimensional visualization of intravascular tumor cells in mice. *Trends Mol. Med.* **7**, 377 (2001).
99. Cummings, R. D. Selectins. in *Essentials of Glycobiology* (eds Varki, A. *et al.*) 391–415 (Cold Spring Harbor Laboratory Press, New York, 1999).
100. Kim, Y. J., Borsig, L., Varki, N. M. & Varki, A. P-selectin deficiency attenuates tumor growth and metastasis. *Proc. Natl Acad. Sci. USA* **95**, 9325–9330 (1998).
Describes the observation that P-selectin is important for the formation of tumour-platelet emboli during metastasis, and facilitates haematogenous dissemination of mucin-producing carcinoma cells.
101. Borsig, L., Wong, R., Hynes, R. O., Varki, N. M. & Varki, A. Synergistic effects of L- and P-selectin in facilitating tumor metastasis can involve non-mucin ligands and implicate leukocytes as enhancers of metastasis. *Proc. Natl Acad. Sci. USA* **99**, 2193–2198 (2002).
102. Varki, A. Selectin ligands: will the real ones please stand up? *J. Clin. Invest.* **100**, S31– S35 (1997).
103. Nakamori, S. *et al.* Involvement of carbohydrate antigen sialyl Lewis(x) in colorectal cancer metastasis. *Dis. Colon Rectum.* **40**, 420–431 (1997).
104. Baldus, S. E. *et al.* Histopathological subtypes and prognosis of gastric cancer are correlated with the expression of mucin-associated sialylated antigens: Sialosyl-Lewis(a), Sialosyl-Lewis(x) and sialosyl-Tn. *Tumour Biol.* **19**, 445–453 (1998).
105. Nakamori, S. *et al.* Molecular mechanism involved in increased expression of sialyl Lewis antigens in ductal carcinoma of the pancreas. *J. Exp. Clin. Cancer Res.* **18**, 425–432 (1999).
106. Muller, A. *et al.* Involvement of chemokine receptors in breast cancer metastasis. *Nature* **410**, 50–56 (2001).
107. Almofti, A. *et al.* The clinicopathological significance of the expression of CXCR4 protein in oral squamous cell carcinoma. *Int. J. Oncol.* **25**, 65–71 (2004).
108. Crocker, P. R. Siglecs: sialic-acid-binding immunoglobulin-like lectins in cell-cell interactions and signalling. *Curr. Opin. Struct. Biol.* **12**, 609–615 (2002).
109. Brinkman-Van der Linden, E. C. & Varki, A. New aspects of siglec binding specificities, including the significance of fucosylation and of the sialyl-Tn epitope. Sialic acid-binding immunoglobulin superfamily lectins. *J. Biol. Chem.* **275**, 8625–8632 (2000).
110. Liu, F. T. & Rabinovich, G. A. Galectins as modulators of tumour progression. *Nature Rev. Cancer* **5**, 29–41 (2005).
111. Pearlstein, E., Salk, P. L., Yogeeswaran, G. & Karpatkin, S. Correlation between spontaneous metastatic potential, platelet-aggregating activity of cell surface extracts, and cell surface sialylation in 10 metastatic-variant derivatives of a rat renal sarcoma cell line. *Proc. Natl Acad. Sci. USA* **77**, 4336–4339 (1980).
112. Fuster, M. M., Brown, J. R., Wang, L. & Esko, J. D. A disaccharide precursor of sialyl Lewis X inhibits metastatic potential of tumor cells. *Cancer Res.* **63**, 2775–2781 (2003).
113. Al-Mehdi, A. B. *et al.* Intravascular origin of metastasis from the proliferation of endothelium-attached tumor cells: a new model for metastasis. *Nature Med.* **6**, 100–102 (2000).
114. Winn, R. K., Liggitt, D., Vedder, N. B., Paulson, J. C. & Harlan, J. M. Anti-P-selectin monoclonal antibody attenuates reperfusion injury to the rabbit ear. *J. Clin. Invest.* **92**, 2042–2047 (1993).
115. Fukuda, M. N. *et al.* A peptide mimic of E-selectin ligand inhibits sialyl Lewis X-dependent lung colonization of tumor cells. *Cancer Res.* **60**, 450–456 (2000).
116. Kaila, N. & Thomas, B. E. t. Design and synthesis of sialyl Lewis(x) mimics as E- and P-selectin inhibitors. *Med. Res. Rev.* **22**, 566–601 (2002).
117. Leppanen, A. *et al.* A novel glycosulfopeptide binds to P-selectin and inhibits leukocyte adhesion to P-selectin. *J. Biol. Chem.* **274**, 24838–24848 (1999).
118. Varki, N. M. & Varki, A. Heparin inhibition of selectin-mediated interactions during the haematogenous phase of carcinoma metastasis: rationale for clinical studies in humans. *Semin. Thromb. Hemost.* **28**, 53–66 (2002).
119. Orner, B. P., Derda, R., Lewis, R. L., Thomson, J. A. & Kiessling, L. L. Arrays for the combinatorial exploration of cell adhesion. *J. Am. Chem. Soc.* **126**, 10808–10809 (2004).
120. Brown, J. R., Fuster, M. M., Whisenant, T. & Esko, J. D. Expression patterns of α 2, 3-sialyltransferases and α 1, 3-fucosyltransferases determine the mode of sialyl Lewis X inhibition by disaccharide decoys. *J. Biol. Chem.* **278**, 23352–23359 (2003).
121. Sarkar, A. K., Rostand, K. S., Jain, R. K., Matta, K. L. & Esko, J. D. Fucosylation of disaccharide precursors of sialyl LewisX inhibit selectin-mediated cell adhesion. *J. Biol. Chem.* **272**, 25608–25616 (1997).
Demonstrates the ability of disaccharide decoys of glycosylation to alter expression of the glycan selectin ligand SLe^x on the surface of lymphoma cells. This is a novel method to reduce the levels of

© 2005 Nature Publishing Group

selectin ligands on a range of carcinomas, thereby altering the metastatic potential of tumour cells.
122. Qian, F., Hanahan, D. & Weissman, I. L. L-selectin can facilitate metastasis to lymph nodes in a transgenic mouse model of carcinogenesis. *Proc. Natl Acad. Sci. USA* **98**, 3976–3981 (2001).
123. Renkonen, J., Paavonen, T. & Renkonen, R. Endothelial and epithelial expression of sialyl Lewis(x) and sialyl Lewis(a) in lesions of breast carcinoma. *Int. J. Cancer* **74**, 296–300 (1997).
124. Glithero, A. *et al.* Crystal structures of two H-2Db/glycopeptide complexes suggest a molecular basis for CTL cross-reactivity. *Immunity* **10**, 63–74 (1999).
125. Ibrahim, N. K. & Murray, J. L. Clinical Development of the STn–KLH Vaccine (Theratope(R)). *Clin. Breast Cancer* **3** (Suppl. 4), 139– 143 (2003).
The authors describe the development of a unique tumour vaccine by conjugating a synthetic STn epitope to a high-molecular-weight carrier, KLH. The STn–KLH vaccine generates a humoral and cellular immune response, and its safety was confirmed in the ongoing Phase III trial described in the article.
126. Holmberg, L. A., Oparin, D. V., Gooley, T. & Sandmaier, B. M. The role of cancer vaccines following autologous stem cell rescue in breast and ovarian cancer patients: experience with the sTn–KLH vaccine (Theratope(R)). *Clin. Breast Cancer* **3** (Suppl. 4), 144–151 (2003).
127. de Kleijn, E. M. & Punt, C. J. Biological therapy of colorectal cancer. *Eur. J. Cancer* **38**, 1016–1022 (2002).
128. Monzavi-Karbassi, B., Cunto-Amesty, G., Luo, P. & Kieber-Emmons, T. Use of surrogate antigens as vaccines against cancer. *Hybrid Hybridomics* **21**, 103–109 (2002).
129. Lemieux, G. A. & Bertozzi, C. R. Modulating cell surface immunoreactivity by metabolic induction of unnatural carbohydrate antigens. *Chem. Biol.* **8**, 265–275 (2001).
Sialic acid is often expressed by tumour-associated oligosaccharide antigens; the authors explored whether intercepting the sialic acid biosynthetic pathway with unnatural precursors might modulate the immunogenicity of targeted tumour cells. Antibodies generated against the unnatural sialic acid directed complement-mediated lysis of tumour cells.
130. Wolfl, M., Batten, W. Y., Posovszky, C., Bernhard, H. & Berthold, F. Gangliosides inhibit the development from monocytes to dendritic cells. *Clin. Exp. Immunol.* **130**, 441–448 (2002).
131. Caldwell, S. *et al.* Mechanisms of ganglioside inhibition of APC function. *J. Immunol.* **171**, 1676–1683 (2003).
132. Freeze, H. H., Sampath, D. & Varki, A. α- and β-xylosides alter glycolipid synthesis in human melanoma and Chinese hamster ovary cells. *J. Biol. Chem.* **268**, 1618–1627 (1993).
133. Weiss, M., Hettmer, S., Smith, P. & Ladisch, S. Inhibition of melanoma tumor growth by a novel inhibitor of glucosylceramide synthase. *Cancer Res.* **63**, 3654–3658 (2003).
134. Ragupathi, G. *et al.* Consistent antibody response against ganglioside GD2 induced in patients with melanoma by a GD2 lactone-keyhole limpet hemocyanin conjugate vaccine plus immunological adjuvant QS-21. *Clin. Cancer Res.* **9**, 5214–5220 (2003).
135. Slovin, S. F. *et al.* Carbohydrate vaccines in cancer: immunogenicity of a fully synthetic globo H hexasaccharide conjugate in man. *Proc. Natl Acad. Sci. USA* **96**, 5710–5715 (1999).
136. Pagnan, G. *et al.* Delivery of c-myb antisense oligodeoxynucleotides to human neuroblastoma cells via disialoganglioside GD(2)-targeted immunoliposomes: antitumor effects. *J. Natl Cancer Inst.* **92**, 253–261 (2000).
137. Nakagoe, T. *et al.* Difference in prognostic value between sialyl Lewis(a) and sialyl Lewis(x) antigen levels in the preoperative serum of gastric cancer patients. *J. Clin. Gastroenterol.* **34**, 408–415 (2002).
138. Nakagoe, T. *et al.* Preoperative serum level of CA19–9 predicts recurrence after curative surgery in node-negative colorectal cancer patients. *Hepatogastroenterology* **50**, 696–699 (2003).
139. Satoh, H. *et al.* Predictive value of preoperative serum sialyl Lewis X-i antigen levels in non-small cell lung cancer. *Anticancer Res.* **18**, 2865–2868 (1998).
140. Seelenmeyer, C., Wegehingel, S., Lechner, J. & Nickel, W. The cancer antigen CA125 represents a novel counter receptor for galectin-1. *J. Cell Sci.* **116**, 1305–1318 (2003).
141. Hayashi, N. *et al.* Association between expression levels of CA 19–9 and *N*-acetylglucosamine-β;1, 3-galactosyltransferase 5 gene in human pancreatic cancer tissue. *Pathobiology* **71**, 26–34 (2004).
142. Schroeder, J. A., Adriance, M. C., Thompson, M. C., Camenisch, T. D. & Gendler, S. J. MUC1 alters β-catenin-dependent tumor formation and promotes cellular invasion. *Oncogene* **22**, 1324–1332 (2003).
143. Luchansky, S. J., Goon, S. & Bertozzi, C. R. Expanding the diversity of unnatural cell-surface sialic acids. *Chembiochem.* **5**, 371–374 (2004).
144. Saxon, E. & Bertozzi, C. R. Cell surface engineering by a modified Staudinger reaction. *Science* **287**, 2007–2010 (2000).
145. Dwek, R. A., Butters, T. D., Platt, F. M. & Zitzmann, N. Targeting glycosylation as a therapeutic approach. *Nature Rev. Drug Discov.* **1**, 65–75 (2002).
146. Brown, J. R., Fuster, M. M. & Esko, J. D. in *Carbohydrate Based Drug Discovery*, Vol. 2 (ed. Wong, C.-H.) 883–898 (Wiley VCH, Weinheim, 2003).
147. Fritz, T. A., Lugemwa, F. N., Sarkar, A. K. & Esko, J. D. Biosynthesis of heparan sulfate on β-D-xylosides depends on aglycone structure. *J. Biol. Chem.* **269**, 300–307 (1994).
148. Dimitroff, C. J., Sharma, A. & Bernacki, R. J. Cancer metastasis: a search for therapeutic inhibition. *Cancer Invest.* **16**, 279–290 (1998).
149. Prescher, J. A., Dube, D. H. & Bertozzi, C. R. Chemical remodelling of cell surfaces in living animals. *Nature* **430**, 873–877 (2004).
150. Takenaka, Y., Fukumori, T. & Raz, A. Galectin-3 and metastasis. *Glycoconj. J.* **19**, 543–549 (2004).
151. Kojima, N. & Hakomori, S. Cell adhesion, spreading, and motility of GM3-expressing cells based on glycolipid-glycolipid interaction. *J. Biol. Chem.* **266**, 17552–17558 (1991).
152. Pili, R. *et al.* The α-glucosidase I inhibitor castanospermine alters endothelial cell glycosylation, prevents angiogenesis, and inhibits tumor growth. *Cancer Res.* **55**, 2920–2926 (1995).
153. Kleeff, J. *et al.* Stable transfection of a glypican-1 antisense construct decreases tumorigenicity in PANC-1 pancreatic carcinoma cells. *Pancreas* **19**, 281–288 (1999).
154. Miyamoto, S. *et al.* Heparin-binding EGF-like growth factor is a promising target for ovarian cancer therapy. *Cancer Res.* **64**, 5720–5727 (2004).
155. Derksen, P. W. *et al.* Cell surface proteoglycan syndecan-1 mediates hepatocyte growth factor binding and promotes Met signaling in multiple myeloma. *Blood* **99**, 1405–1410 (2002).
156. Mercurio, A. M., Bachelder, R. E., Bates, R. C. & Chung, J. Autocrine signaling in carcinoma: VEGF and the $\alpha_6\beta_4$ integrin. *Semin. Cancer Biol.* **14**, 115–122 (2004).
157. Syrokou, A., Tzanakakis, G. N., Hjerpe, A. & Karamanos, N. K. Proteoglycans in human malignant mesothelioma. Stimulation of their synthesis induced by epidermal, insulin and platelet-derived growth factors involves receptors with tyrosine kinase activity. *Biochimie* **81**, 733–744 (1999).
158. George, D. Targeting PDGF receptors in cancer—rationales and proof of concept clinical trials. *Adv. Exp. Med. Biol.* **532**, 141–151 (2003).
159. Wang, L., Fuster, M. M., Sriranardo, P. & Esko, J. D. Endothelial deficiency of heparan sulphate impairs L-selectin and chemokine mediated neutrophil trafficking during inflammatory responses. *Nature Immunol.* (in the press).

Acknowledgements:
We would like to thank A. Varki for many helpful comments. This work was supported by a Research Career Development Award from the US Department of Veteran's Affairs (to M.F.) and National Institutes of Health (to J.D.E.).

Competing interests statement
The authors declare no competing financial interests.

Online links

DATABASES
The following terms in this article are linked online to:
Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
CD44 | COSMC | E-cadherin | EGF | ERBB2 | FGF2 | glypican-1 | glypican-3 | HB-EGF | IGF1R | IL-8 | MUC1 | MUC16 | MUC4 | TGFβ | VEGF
National Cancer Institute: http://www.cancer.gov/
breast cancer | melanoma | ovarian cancer | pancreatic cancer

FURTHER INFORMATION
Complex Carbohydrate Research Center: http://www.ccrc.uga.edu/
Consortium for Functional Glycomics: http://www.functionalglycomics.org/static/consortium/
Glycobiology journal: http://glycob.oupjournals.org
Glycobiology Research and Training Center: http://grtc.ucsd.edu
National Cancer Institute Glycobiology Resources: http://glycores.ncifcrf.gov
Oxford Glycobiology Institute: http://www.bioch.ox.ac.uk/glycob/
Society for Glycobiology: http://www.glycobiology.org/pub/handler.asp
Access to this interactive links box is free online.

© 2005 Nature Publishing Group