Leaders

Protein glycosylation in cancer biology: an overview

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Introduction

Glycosylation is one of the most frequently occurring post-translational modifications of proteins. The presence of an oligosaccharide moiety in soluble and membrane bound proteins improves solubility in water, contributes to the proper orientation of the molecule, protects it from proteases and, in some cases, is required for efficient intracellular transport. Some oligosaccharide sequences may also mediate highly specific molecular and cellular recognition. In the first section of this review the biochemistry of protein glycosylation is summarised briefly; the second section deals with cancer related oligosaccharide changes and invasive properties of cancer cells, with special emphasis on experimental systems; the third section focuses on specific oligosaccharide structural alterations in human cancers.

Biosynthesis of glycoprotein oligosaccharides

The biosynthesis of glycoprotein oligosaccharides (for a detailed review see^{1,2}), is catalysed by glycosyltransferases, a family of 100 or more enzymes which transfer a sugar residue from a nucleotide-sugar donor (for example, GDP-fucose, UDP-galactose and CMP-sialic acid) to an acceptor which can be a sugar, an amino acid or a lipid. Glycosyltransferases are classified on the basis of the sugar they transfer (for example, fucosyltransferases, galactosyltransferases, sialyltransferases). Moreover, members of each glycosyltransferase family are distinguished on the basis of the structure they recognise as an acceptor and of the isomeric linkage they form (for example, α 2,3 and α 2,6 sialyltransferases catalyse the linkage of sialic acid to either the third or the sixth oxydril group of the penultimate sugar residue). The biosynthesis of a given glycosidic structure is controlled mainly at the level of expression of the cognate glycosyltransferase. Glycoprotein bound oligosaccharides fall into two well defined categories: those linked to the amidic nitrogen of asparagine (*N*-linked) and those linked to oxydril side group of serine or threonine (*O*-linked).

BIOSYNTHESIS OF N-LINKED CHAINS

This process occurs through several discrete steps (fig 1). In the rough endoplasmic reticu-

lum (RER) an oligosaccharide comprised of two *N*-acetylglucosamine (GlcNAc), nine mannose and three glucose residues is synthesised on a lipid carrier, the dolichol-phosphate, by the sequential action of the corresponding glycosyltransferases (fig 1A). This oligosaccharide is then transferred en bloc to an asparagine residue of the nascent polypeptide chain by the action of a multienzymatic complex, the oligosaccharyltransferase. Oligosaccharides containing five or more mannose residues are referred to as "high-mannose types". After the transfer to protein the three glucose residues and four of the nine mannose residues are "trimmed" by specific glycosidases. The "trimmed" oligosaccharides, comprising five mannose and two GlcNAc residues (fig 1B), may then act as acceptors for GlcNAc transferase I, located in the Golgi apparatus (fig 1C). The addition of the first GlcNAc residue represents the first event of the conversion of the oligosaccharide from the "high-mannose" to the "complex type"; two other mannose residues are subsequently removed by Golgi mannosidase II and other GlcNAc and galactose residues are added (fig 1D). The resulting branched structure may be elongated by the sequential addition of galactose, fucose and sialic acid. A typical tetra-branched, fully sialylated "complex type" glycan is shown in fig 1E. These structures are comprised of an invariant "core" portion containing three mannose and two GlcNAc residues and two to five antennae.

BIOSYNTHESIS OF O-LINKED CHAINS

N-acetylgalactosamine (GalNAc) is the first sugar residue to be linked to serine or threonine. Without further elongation, this structure forms the Tn antigen (fig 2). Elongation with galactose linked to the *O*-3 position creates the core 1 structure, while the presence of GlcNAc linked β 1,6- or β 1,3- to GalNAc forms core 2 and 3 structures, respectively (fig 2). These three structures are often further elongated by the addition of other galactose, GlcNAc and GalNAc residues, and terminated by fucose or sialic acid, or both. The unsubstituted core 1 structure represents the Thomsen-Friedenreich (or T) epitope. Substitution of the first GalNAc in the *O*-6 position with sialic acid creates the sialyl-Tn antigen, which does not undergo further elongation. It is gen-

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erally agreed that the first step of O-glycosylation occurs in *cis* Golgi, while the addition of other sugars takes place in more distal compartments.

Aberrant glycosylation in cancer cells

Generally, the most frequently described cancer related changes in the pattern of glycosylation include the synthesis of highly branched and heavily sialylated glycans,³ the premature termination of biosynthesis, resulting in the expression of uncompleted forms, and the re-expression of glycosidic antigens of fetal type.⁴ In many cases the formation of these aberrant structures depends on altered regulation of one or more key glycosyltransferases; the molecular basis of this phenomenon remains obscure in other cases.⁵ In experimental systems the relation between altered glycosylation and cancer biology has been studied using one of two approaches: the first involves the modification of the glycosylation pattern of cancer cell membranes by means of glycosylation inhibitors or glycosidase treatments; the second is based on the study of subpopulations of cancer cells selected for a given phenotype—for instance, increased or reduced metastatic ability or resistance to a given lectin.

MODIFICATION OF GLYCOSYLATION PATTERN

There are several inhibitors of specific steps of N-linked biosynthesis. Tunicamycin blocks the first step of N-glycosylation, giving rise to the production of proteins devoid of N-linked chains. Castanospermine inhibits glucosidase I, the first “trimming” enzyme, causing the formation of glycoproteins bearing high mannose N-linked chains terminating with glucose (fig 1A). Swainsonine is an inhibitor of Golgi mannosidase II, the last “trimming” enzyme and induces the formation of “hybrid type” glycans, similar to the structure shown in fig 1C, comprised of five mannose residues and one complex type antenna, usually formed by GlcNAc, galactose and sialic acid. Treatment of B16 murine melanoma cells with tunicamycin, castanospermine or swainsonine results in a dramatic reduction in the number of pulmonary metastases in syngeneic mice and in

reduced retention of drug treated cells in the target organ.^{6–8} Consistent results have been obtained with L-1 mouse sarcoma cells.⁹ In mouse mammary tumour cells, however, tumour and metastasis formation was inhibited on treatment with tunicamycin, but not with swainsonine.¹⁰ Swainsonine inhibits the growth of human cell lines *in vivo* at the site of inoculation^{11 12} and metastasis formation,^{13 14} even when administered to host animals systemically. Two mechanisms have been proposed to explain such effects. First, combination treatment with swainsonine and interferon- α 2 enhances the activity of the interferon inducible enzyme 2'5'-oligoadenylate synthetase, suggesting that swainsonine may potentiate the antiproliferative effect of interferon.^{11 15} Second, treatment with swainsonine increases natural killer (NK) cell activity of host animals two to threefold and swainsonine dependent reduction in metastasis formation is not observed in NK cell depleted animals.¹³ Swainsonine is currently under consideration as an antineoplastic agent in a phase I clinical study.¹⁶ The sialyltransferase specific inhibitor KI-8110 has facilitated the investigation of the role of sialic acid in the regulation of the metastatic cascade. Because of its negative electric charge and as it is frequently present at the terminal non-reducing end of glycoprotein oligosaccharides, sialic acid has long been suspected of playing a key role in mediating biological recognition events, including those responsible for invasive tumour growth. Treatment of murine¹⁷ and human¹⁸ colon cancer cell lines with KI-8110 results in a significant reduction in sialic acid on the cell surface and in a dramatic reduction in lung and liver metastasis formation. Enzymatic removal of sialic acid increases binding of collagen type IV and fibronectin by highly metastatic cell lines.¹⁹ Treatment with KI-8110, however, does not affect adhesion to these substrates, but decreases the ability of tumour cells to induce platelet aggregation.²⁰ This suggests that sialic acid may exert its effect on metastasis formation by controlling the formation of aggregates of tumour cells and platelets.

Figure 1 Simplified representation of the N-linked chain biosynthetic pathway. See text for details. Glc = glucose; Man = mannose; Gal = galactose; GlcNAc = N-acetylglucosamine; SA = sialic acid. Glycans containing five to nine mannose residues (structures A–C), may also be retained by mature glycoproteins. The number of branches (antennae) in complex glycans usually ranges from 2 to 4. Sialic acid may be linked to galactose either via α 2,3- or α 2,6-linkages. The number of sialic acid residues usually ranges between 0 and 4 (structure E). The core pentasaccharide is boxed in E.

USE OF SELECTED MUTANTS

Many authors have studied subpopulations of cells selected for resistance to wheat germ agglutinin (WGA), a lectin with a broad specificity for sialic acid, terminal GlcNAc and poly-*N*-acetylglucosaminic units (that is, repeating Gal β 1,4GlcNAc units).²¹ In 1977, Tao and Burger²² reported the isolation of WGA resistant clones of mouse melanoma cells displaying a dramatic reduction in metastatic ability. In 1981, Yogeewaran and Salk²³ reported that the ability of murine tumour cells to metastasise spontaneously from subcutaneous sites of injection was positively correlated with substitution of GalNAc and galactose by sialic acid (that is, the larger the number of GalNAc and galactose residues "covered" by sialic acid, the greater the number of metastatic cells). Studies on WGA resistant variants and revertants of the murine melanoma cell line MDAY-D2 indicate that reduced expression of surface sialic acid is associated with a poorly metastatic phenotype and increased binding to collagen type IV and fibronectin.²⁴⁻²⁵ The mutant WGA resistant cells lack large, sialic acid bearing *N*-linked glycans, with poly-*N*-acetylglucosaminic units (normally present in parent WGA sensitive cells) and accumulate truncated glycans.²⁶⁻²⁷ The relation between WGA resistance and reduced metastatic ability has been confirmed for different human and murine cancer cell lines.²⁸⁻³¹ Moreover, the ability of T cell hybridoma clones to invade primary cultures of hepatocytes and to metastasise in syngeneic mice is positively correlated with reduced binding of lectins specific for penultimate sugar residues, which indicates a high degree of sialic acid substitution.³² However, other reports suggest that this relation may be less direct. Subpopulations of colon cancer cells expressing a more aggressive phenotype bear a higher number of cell surface sialic acid residues but this results in increased rather than decreased binding to collagen type IV and fibronectin.³³ Other investigators failed to detect any difference in the amount of sialic acid released by clones of the B16 murine melanoma cell line with differing metastatic potentials. Rather, they found a correlation between metastatic potential and the number of terminal galactose residues.³⁴⁻³⁵ The involve-

ment of terminal galactose residues in the metastatic cascade is supported, firstly, by the observation that in vitro binding of a glycosylation mutant of the lymphoreticular cell line MDAY-D2, lacking terminal sialic acid and galactosyl residues, to endothelial cells on galactosylation is increased by exogenous galactosyltransferase³⁶; and, secondly, that the capacity of adrenal carcinoma cells to invade a reconstituted basement membrane in vitro is correlated with the level of galactosylation of cell surface glycoproteins catalysed by a cell surface galactosyltransferase.³⁷

Although sometimes conflicting, the data outlined here indicate that the absence of or a reduction in the number of fully processed, sialic acid-bearing *N*-linked glycans reduces the invasiveness of cancer cells, probably by altering their adhesion properties or by increasing their susceptibility to the host's immune system. However, on the one hand, it should be remembered that some inhibitors of glycosylation are cytotoxic, that their effect is reversible and is not specific for particular oligosaccharide structures. On the other hand, cells selected for a given phenotype may differ from the original cell population, not only for the chosen phenotype but also for many other properties. Thus, any causal relation between altered glycosylation and the phenotype of cancer cells should be considered with great care.

Oligosaccharide structures specifically involved in cancer biology

Changes in the following oligosaccharide structures have often been associated with human cancer and are important because: (1) they may be related to a precise stage of the disease and their detection with lectins or monoclonal antibodies may provide useful diagnostic or prognostic information, or both; and (2) in many cases they contribute directly to cancer biology.

POLYSIALIC ACID

Polysialic acid (PSA) is a linear polymer formed by sialic acid residues usually linked through α 2,8 bonds. This is the only known instance where sialic acid occurs as an internal sugar. In mammals PSA occurs virtually only on *N*-linked chains of the neural cell adhesion molecule (N-CAM) and on the α subunit of sodium channels in the brain. Its pattern of expression is developmentally regulated: the number of sialic acid residues arranged as PSA on N-CAM is around 10 in fetal rat brain and around two to three in the adult.³⁸ The maturation dependent shift from the expression of the high to the low PSA bearing form of N-CAM plays a pivotal role in controlling the strength of cell-cell and cell-matrix interactions.³⁹⁻⁴⁰ High PSA bearing N-CAM is expressed by several human fetal tissues and re-expressed by the corresponding neoplasms. Indeed, its presence has been reported in malignant tumours of neuroectodermal origin, such as medulloblastomas and neuroblastomas,⁴¹ and in neuroblastoma cell lines,⁴²⁻⁴³ but not in ependymomas and gliomas.⁴¹ Remark-

Figure 2 Commonly occurring biosynthetic pathways of O-linked chain biosynthesis. Abbreviations as in fig 1; GalNAc = *N*-acetylglucosamine; β 1,3gal-T = β 1,3-galactosyltransferase (Gal to GalNAc); β 1,6GlcNAc-T = β 1,6-*N*-acetylglucosaminyltransferase (GlcNAc to GalNAc); β 1,3GlcNAc-T = β 1,3-*N*-acetylglucosaminyltransferase (GlcNAc to GalNAc); α 2,6SA-T = α 2,6-sialyltransferase (SA to GalNAc).

ably, polysialic acid chains longer than 55 residues have been described in CHP-134 neuroblastoma cells.⁴³ It should be emphasised that the presence of the long chains of PSA may have an enormous effect on the strength of homophilic binding between N-CAM molecules on different cells. High PSA is expressed by small cell lung carcinoma (SCLC),^{42,44} a cancer of neuroendocrine origin with a very poor prognosis, but not by carcinoids or other lung tumours. High PSA expression in SCLC correlates with reduced cell-cell adherence, greater clonogenic ability in semisolid media and a significantly higher metastatic ability in nude mice.⁴⁵ Among thyroid tumours, high PSA is expressed by medullary carcinomas and has been proposed as a useful marker to distinguish medullary carcinomas from other thyroid tumours.⁴⁶ The presence of high PSA in the mesodermally derived Wilms tumour⁴⁷ indicates that the expression of these structures is not restricted to tissues of neuroectodermal origin.

SIALIC ACID α 2,6-LINKED TO GALACTOSE

In about 90% of colon cancer specimens the activity of the enzyme catalysing the addition of sialic acid in α 2,6-linkage to galactose residues of N-linked chains (α 2,6ST) is increased.⁴⁸ This results in augmented binding of the *Sambucus nigra* lectin (SNA), specific for α 2,6-linked sialic acid, to histological sections of the vast majority of grade I and II colon carcinomas^{49,50}; the few cases of grade III carcinoma examined were poorly or non-reactive.⁵⁰ Among benign lesions, strong SNA reactivity is expressed only by polyps with severe dysplasia.^{49,50} A very similar pattern of expression was observed in an histochemical study utilising the lectin from *Trichosanthes japonica*, specific for sialic acid α 2,6-linked to Gal β 1,4GlcNAc.⁵¹ These data, together with the differentiation dependent expression of α 2,6ST shown by rat^{52,53} and human⁵⁴ intestinal cells, suggest that a high level of α 2,6-sialylation in the colonic cell is restricted to a specific degree of tissue differentiation. Enhanced α 2,6-sialylation of galactose in colon cancer has also been found to be associated with glycolipids.^{55,56} In fibroblasts transfection with activated ras oncogenes results in the enhancement of α 2,6ST activity and mRNA expression,^{57,58} and in an increased invasive potential.⁵⁹ A role for this modification in colon cancer progression is suggested by several observations. Metastatic specimens of colorectal cancer express an α 2,6ST at even higher levels than primary tumours.⁶⁰ Subpopulations of the adherent colon carcinoma cell line SW948, which is devoid of α 2,6ST activity, selected for their ability to grow in non-adherent conditions, express high levels of α 2,6ST activity,⁶¹ suggesting that this sialyl-linkage contributes to reduced colon cancer cell adhesion. Murine colon cancer cell lines selected for the ability to metastasise in the liver after caecal injection, express two to three times more SNA binding proteins than the poorly metastatic parental cell line.⁶² Variants of the murine colon cancer cell line MCA-38 with high liver colonising potential express an

α 2,6ST mRNA level about fourfold higher than low liver colonising variants.⁶³ Human colon cancer cell lines grown as xenografts in nude mice and the xenograft derived cell lines expressed an α 2,6ST activity significantly higher than that of the parental cell line.⁶⁴ By contrast, a mutant of the murine melanoma cell line MDAY-D2, with increased α 2,6ST activity, has a reduced metastatic ability.⁶⁵ The recent establishment of a panel of human colon cancer cell lines differing only in their level of α 2,6ST expression, obtained by transfection with α 2,6ST cDNA, should provide more direct information on the role of α 2,6-linked sialic acid in colon cancer progression.⁶⁶

β 1-6 BRANCHING

One of the most important cancer related changes is increased β 1,6 branching of N-linked chains. This modification involves the presence of an antenna whose first GlcNAc residue is β 1,6-linked to a core mannose residue (fig 1D) and has been directly associated with metastatic potential. Transfection of NIH 3T3 cells with DNA from neuroblastoma and bladder carcinoma cell lines containing activated ras oncogenes results in enlarged glycoprotein oligosaccharides, detected by a shift in the gel filtration profile.^{67,68} Glycans from transfected cells showed an increased affinity to leucoagglutinin (L-PHA), a lectin specific for N-linked chains containing a β 1,6-linked antenna.⁶⁷ This modification may also be caused by transformation with both DNA⁶⁹ and RNA⁷⁰ tumour viruses and by transfection with the v-fps/fes oncogene, which encodes a cytoplasmic tyrosine kinase,⁷¹ but not by transfection with the nuclear oncogene myc.⁷¹ A direct association between increased β 1,6-branching and metastatic potential is suggested by several studies. Mutants of MDAY-D2 cells selected for L-PHA resistance display a tumorigenic potential similar to that of the parental cells, but their metastatic potential is dramatically reduced.⁷² The activity of GlcNAc transferase V, the enzyme responsible for β 1,6-branching, is reduced, as is binding of L-PHA to 130 kilodalton glycoproteins. Transfection of the tumorigenic, non-metastatic mammary adenocarcinoma cell line SP1 with activated T24H-ras, but not with non-activated c-H-ras, results in the expression of the metastatic phenotype and a concomitant increase in binding of L-PHA to 130 kilodalton glycoproteins.⁷² L-PHA reactive oligosaccharides are preferentially expressed by the lysosomal associated membrane glycoprotein 1 (LAMP-1), a glycoprotein found largely in lysosomal membranes and also in the plasma membrane.^{73,74} It has been suggested that the presence of β 1-6 branched N-linked chains facilitates the invasion of basement membranes.⁷⁵ Clinical studies revealed an increased level of L-PHA reactivity in tissue sections of human cancers. In oesophageal carcinomas L-PHA positive cells are distributed predominantly on the outer surface of the tumour, adjacent to the surrounding tissue.⁷⁶ All breast carcinomas and epithelial hyperplasia with atypia showed increased staining for L-PHA compared with fibroad-

enomas and hyperplasia without atypia.⁷⁷ In O-linked chains the presence of a β 1,6-linked GlcNAc residue (in this case to the peptide linked GalNAc; core 2 structure) has also been associated with metastatic behaviour and the activity of the enzyme catalysing this reaction (core 2 β 1,6GlcNAc transferase) is raised in metastatic murine tumour cell lines,⁷⁸ and also in human acute myeloblastic and chronic myelogenous leukaemia cells.⁷⁹ O-linked glycans containing core 2 structures are present, linked to the major glycoprotein leukosialin, on the surface of activated T lymphocytes and in acute T lymphocytic leukaemia cells.^{80 81} By contrast, in human colon core 2 β 1,6 GlcNAc transferase activity decreases during progression to cancer.^{82 83}

LACTO SERIES CHAINS

As shown in fig 3, galactose may be linked to GlcNAc either through a β 1,3 or through a β 1,4 linkage, forming the two basic units for linear type 1 and type 2 lacto series chains, respectively. Repetition of the two basic units form the extended lacto series chains, frequently found in N- and O-linked glycans of glycoproteins and in glycolipids. When extended type 2 chains, which are referred to as poly lactosaminic, are linearly arranged, the “i” antigen is formed. The presence of a GlcNAc β 1,6-linked to galactose provides a branching point which, with further elongation, forms the

“I” antigen (fig 3). The key enzymes controlling the elongation (the i antigen) or branching (the I antigen) of the poly lactosaminic chain are a β 1,3GlcNAc transferase and a β 1,6GlcNAc transferase, respectively.⁸⁴⁻⁸⁷ Several observations indicate that expression of the Ii antigens is regulated onco-developmentally. Human fetal erythrocytes express the i antigen, whereas after birth, biosynthesis shifts toward production of the I antigen.⁸⁷ Alterations in Ii antigen expression have been reported in pancreatic and lung cancers,^{88 89} while a reduction in overall poly lactosaminoglycan expression occurs on differentiation of promyelocytic leukaemic cells (HL-60)⁹⁰ and human colon cancer cells (CaCo-2).⁹¹ Extended lacto series chains are important not only because they are cancer related antigens but also because they provide the backbone structure for fucosyl substitutions, which give rise to ABH and Lewis antigens, and for sialyl substitutions (fig 3) (see later). In both N- and O-linked chains poly lactosaminic sequences are mounted preferentially on β 1,6-linked GlcNAc residues^{70 78 84}; therefore, the expression of poly lactosaminic chains seems to be controlled by the degree of β 1,6-branching.

ABH HISTO-BLOOD GROUP ANTIGENS

ABH antigens are carried by both glycoproteins and glycolipids on red blood cells, endothelial and epithelial cells of many tissues and secretions. Detailed descriptions of the cancer related alterations of these histo-blood group antigens have been published previously.⁹²⁻⁹⁶ Aberrant expression of ABH antigens in tumours encompasses the following: (1) deletion of an antigen normally present in the corresponding adult tissue, with concomitant accumulation of the precursor sugar chain; (2) re-expression of fetal antigens normally absent in adult tissue; and (3) “incompatible” antigen expression which means—for example, that an individual expressing the B or O phenotype on erythrocytes may carry the A antigen on colon cancer cells. Whereas the first and second modifications may be generated simply by deregulated expression of one or more of the glycosyltransferases involved in the biosynthesis of the antigen, the third requires a more complex explanation. Indeed, A and B alleles are expressed codominantly and the allele encoding A GalNAc transferase is not present in B or O individuals. The genetic and biochemical basis of this phenomenon is largely unknown. However, it should be remembered that the A and B transferases differ only by four amino acid substitutions⁹⁷ and that under some circumstances the B galactosyltransferase may also transfer GalNAc.⁹⁸ Screening of gastric cancer specimens from blood group O individuals with a monoclonal antibody directed against A-transferase revealed the presence, in 10% of the cases, of the transferase molecule and the corresponding enzyme activity.⁹⁹ ABH antigens are expressed by fetal human colon according to the individual’s blood type. After birth, the expression of A, B and H becomes restricted to the proximal colon.¹⁰⁰ In colon cancer this

Basic unit of type 1 chain	Gal β 1,3GlcNAc
Basic unit of type 2 chain	Gal β 1,4GlcNAc
i antigen	(Gal β 1,4GlcNAc β 1,3Gal β 1,4GlcNAc) _n -R
I antigen	<div style="text-align: center;">GlcNAcβ1,3Galβ1,4GlcNAc-R Galβ1,4GlcNAcβ1,6</div>
H antigen	<div style="text-align: center;">Galβ1,3(4)GlcNAc-R Fuca1,2</div>
A antigen	<div style="text-align: center;">GalNAcα1,3Galβ1,3(4)GlcNAc-R Fuca1,2</div>
B antigen	<div style="text-align: center;">Galα1,3Galβ1,3(4)GlcNAc-R Fuca1,2</div>
Lewis ^a antigen	<div style="text-align: center;">Galβ1,3GlcNAc-R Fuca1,4</div>
Lewis ^b antigen	<div style="text-align: center;">Galβ1,3GlcNAc-R Fuca1,2 Fuca1,4</div>
Lewis ^x antigen	<div style="text-align: center;">Galβ1,4GlcNAc-R Fuca1,3</div>
Lewis ^y antigen	<div style="text-align: center;">Galβ1,4GlcNAc-R Fuca1,2 Fuca1,3</div>
Sialyl dimeric Lewis ^x	<div style="text-align: center;">SAα2,3Galβ1,4GlcNAcβ1,3Galβ1,4GlcNAc-R Fuca1,3 Fuca1,3</div>

Figure 3 Structure of type 1 and type 2 derived glycosidic antigens. In Lewis antigens the presence of a sialic acid residue linked to galactose generates the corresponding sialyl-Le antigens.

proximal–distal gradient of expression is lost because of enhanced distal expression of these antigens.¹⁰⁰ Other examples of aberrant expression of ABH antigens have been reported in pancreatic cancer,¹⁰¹ hepatocellular carcinoma,¹⁰² bladder and oral carcinoma,^{103–104} and ovarian cancer.¹⁰⁵ In lung cancer the loss of ABH antigens has been positively correlated with metastatic potential and consequently with adverse prognosis.¹⁰⁶ However, this relation was not confirmed in another study.¹⁰⁷

LEWIS ANTIGENS

As shown in fig 3, Lewis antigens are originated by the mono- or di-fucosyl substitution of type 1 or type 2 chains. Termination of the sugar chain with sialic acid forms the corresponding sialyl-Lewis antigen. Aberrant expression of Lewis-type antigens has been reported for many cancers, including those of the lung,¹⁰⁸ colon,^{109–110} stomach,¹¹¹ and kidney.¹¹² These antigens are frequently shed into the blood stream.¹¹³ Sialyl-Le^a, detected by monoclonal antibody N19-9, is a useful marker of pancreatic cancer.¹¹⁴ The interest in the expression of Lewis antigens in cancer increased enormously after the discovery that sialyl-Le^x acts as a ligand for E-selectin (previously known as ELAM-1), a lectin-like cell adhesion molecule expressed on activated endothelial cells.^{115–116} Recently, Bergh *et al* reported that the closely related sialyl-Le^a antigen is also a ligand for E-selectin.¹¹⁷ Moreover, sialyl-Le^x may also be recognised by selectins P and L, two other members of the selectin superfamily.¹¹⁸ E-selectin, the physiological role of which is to mediate leucocyte extravasation at sites of tissue damage or injury, may regulate a key event in metastasis formation, causing the arrest of cancer cells expressing the appropriate ligand (that is, sialyl-Le^x) on endothelial cells. Many observations indicate that sialyl-Le^x/sialyl-Le^a–E selectin interactions contribute fundamentally to the adhesion of cancer cells to endothelium.^{119–122} A study carried out on panels of leukaemia and epithelial cancer cell lines reported that all cell lines of epithelial origin can adhere to vascular endothelium through E-selectin mediated interactions. Adhesion of cells of colonic and pancreatic origin depended mainly on sialyl-Le^a. In those originating in the lung and liver sialyl-Le^x contributed significantly to adhesion. Only three of 12 leukaemia cell lines showed E-selectin mediated adhesion to endothelial cells, exclusively through sialyl-Le^x.¹²¹ The importance of sialyl-Le^x/sialyl-Le^a antigens in the regulation of the metastatic cascade has been confirmed in clinical studies. In a study on colorectal cancer, expression of both antigens was higher in metastatic lesions than in the primary tumours.¹²³ In non-small cell lung cancer, expression of the sialyl-Le^x antigen correlates with a shorter disease-free survival,¹²⁴ while postoperative survival was shorter in patients with tumours expressing both sialyl-Le^a and Le^x antigens and Le^y.¹²⁵ In melanomas sialyl-Le^a expression is correlated with tumour progression.¹²⁶ Sialyl dimeric Le^x, an antigenic variant defined by monoclonal antibody FH6, is

generated by di-fucosyl substitution of an extended type 2 chain (fig 3) and seems to be involved in cancer progression. Expression of this antigen is restricted to poorly differentiated areas in primary colonic tumours¹²⁷ and enhanced in liver metastases.¹²⁸ Colon cancer cells selected for high or low sialyl dimeric Le^x antigen expression display a concomitant high or low ability to invade in vitro reconstituted membranes.¹²⁹ A direct correlation between sialyl dimeric Le^x expression and lymph node metastasis formation has been described for transitional cell carcinoma of the human urinary bladder.¹³⁰

T, TN AND SIALYL-TN ANTIGENS

This group of antigens, whose structure is shown in fig 2, represents truncated forms of O-linked chains, which can be detected by monoclonal antibodies or lectins (*Arachis hypogaea*, PNA for T antigen; *Vicia villosa*, VVA or Helix pomatia, HPA for Tn antigen). In normal tissues these antigens are often present in a “cryptic” form—that is: (1) they are masked by the presence of more distal sugar residues, mainly sialic acid; and (2) they are hidden by nearby elaborate glycan structures. In normal human urothelium—for example, the T antigen is substituted with sialic acid (sialy-T antigen) and is not readily detectable,^{96–131–133} unless the samples are treated with sialidase. Pelvic lymph node metastases were detected only in patients whose primary bladder carcinoma was T-antigen positive.¹³³ In a percentage of bladder tumours the expression of T antigen is increased and has been correlated, in non-invasive tumours, with subsequent invasion.¹³⁴ Other investigators proposed the combined use of T and Tn antigens for estimating the degree of malignancy of bladder carcinoma.¹³⁵ Expression of the Tn epitope, as detected with HPA, is a functional predictor of aggressiveness in breast carcinoma.^{136–138} Colonic tumours are probably the best studied model of T and Tn related antigen expression. Practically, none of these antigens is expressed in normal adult epithelium. T and Tn antigens are weakly expressed by hyperplastic polyps, while T, Tn and sialyl-Tn are expressed by adenomatous polyps and carcinomas.^{95–127–139–142} The biochemical basis of aberrant expression of these antigens in colon cancer is complex and incompletely understood—for example, the activity of α 2,6 sialyltransferase synthesising the sialyl-Tn antigen seems to be decreased rather than increased in tumour tissues.¹⁴³ The reduction in core 3 GlcNAc transferase activity, which normally competes with core 1 galactosyltransferase, reported in colon cancer tissues⁸³ and cell lines,⁸² may contribute to the increased expression of T antigen. Of the three mucin associated antigens, sialyl-Tn seems to be the most reliable tumour marker. Indeed, it is rarely expressed by hyperplastic polyps but in adenomas it is preferentially expressed by the larger and more dysplastic lesions.⁹⁵ In colorectal cancer sialyl-Tn is expressed by 87% of cases, regardless of age, gender, location, Dukes’ stage, degree of differentiation, and

ploidy. Five year survival is 100% for sialyl-Tn negative compared with 73% for sialyl-Tn positive patients.¹⁴⁴ Expression of sialyl-Tn has been proposed, together with tumour ploidy, as the most important variant for predicting disease-free and overall survival.¹⁴⁴ Sialyl-Tn expression is a marker of malignancy and is associated with a poor prognosis in many cancers,¹⁴⁵ including those of the pancreas,^{88 146-148} stomach and oesophagus,¹⁴⁸⁻¹⁵⁰ ovary,¹⁵¹ breast,¹⁵² and endometrium.¹⁵³ In Borrmann type IV gastric carcinoma, sialyl-Tn expression correlates with lymph node and peritoneal dissemination and with a higher proliferative activity, as measured by argyrophilic nuclear organiser region (AgNOR) counts.¹⁵⁴ Recent reports indicate that reactivity of normal colon cancer sections with the sialyl-Tn specific monoclonal antibody TKH2 may be increased up to the levels characteristic of cancer samples by treatments which release *O*-acetyl groups from sialic acid.^{155 156} As *O*-acetylation is a modification of sialic acid that occurs more frequently in normal than in cancerous colon, it is possible that the different reactivities observed with TKH2 antibody reflect the loss of *O*-acetyl substituents by sialic acid rather than an accumulation of the disaccharide. This mechanism does not seem to be operating in cancers of the stomach and pancreas.¹⁵⁵ The possibility that mucins carrying the sialyl-Tn epitope play an important role in cancer biology is indicated by the observation that NK cell mediated lysis of conventional target K562 cells may be inhibited by ovine submaxillary mucin (OSM), a mucin bearing the sialyl-Tn antigen.¹⁵⁷ The close association between Tn and sialyl-Tn antigens and neoplastic transformation prompted some investigators to use such antigens for active immunotherapy. Mice immunised with desialylated OSM (that is, carrying the Tn antigen) were protected when challenged with highly invasive Tn antigen expressing, syngeneic mammary tumour cells.¹⁵⁸ Studies carried out on humans indicate that treatment with a vaccine consisting of partially desialylated OSM or of sialyl-Tn disaccharide chemically linked to keyhole limpet haemocyanin in association with an adjuvant may induce a humoral immune response against these tumour associated antigens.^{159 160} However, sialyl-Tn positivity is related, either directly or indirectly, to resistance to adjuvant chemotherapy in patients with breast cancer.¹⁶¹

Concluding remarks

Since early observations in the late 1970s, suggesting a role for complex carbohydrates in cancer biology, the contribution of some cancer related oligosaccharide sequences to the expression of an invasive phenotype has been established. The expression of some of these structures shows a strict correlation with tumour aggression and their detection by lectins or monoclonal antibodies is useful for the clinical management of patients with cancer. The use of glycosylation inhibitors, such as swainsonine, as antineoplastic agents or attempts to use the sialyl-Tn epitope for active anticancer immunotherapy are promis-

ing new approaches for the treatment of cancer. The goal for the next few years will be the elucidation of the precise molecular interactions involving carbohydrate chains in the hope that this will facilitate the design of drugs directed against specific steps of cancer progression.

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