Identification of Asparagine-linked Oligosaccharides Involved in Tumor Cell Adhesion to Laminin and Type IV Collagen

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ABSTRACT MDW4, a wheat germ agglutinin-resistant nonmetastatic mutant of the highly metastatic murine tumor cell line called MDAY-D2 has previously been shown to attach to fibronectin and type IV collagen, whereas MDAY-D2 and phenotypic revertants of MDW4 attached poorly to these substrates. The increased adhesiveness of the mutant cells appeared to be closely related to a lesion in cell surface carbohydrate structures. In an effort to identify the carbohydrates involved in cell attachment, glycopeptides isolated from mutant and wild-type cells as well as from purified glycoproteins were tested for their ability to inhibit the attachment of MDW4 cells to plastic surfaces coated with fibronectin, laminin, or type IV collagen. The addition of mannose-terminating glycopeptide to the adhesion assay inhibited MDW4 cell attachment to type IV collagen. In contrast, a sialylated poly N-acetyllactosamine-containing glycopeptide, isolated from wheat germ agglutinin-sensitive MDAY-D2 cells but absent in MDW4 cells, inhibited MDW4 attachment to laminin. None of the glycopeptides used in this study inhibited attachment of MDW4 cells to fibronectin-coated plastic. Peptide N-glycosidase treatment of the cells to remove surface asparagine-linked oligosaccharides inhibited MDW4 adhesion to type IV collagen, but not to laminin, and the same treatment of the wheat germ agglutinin-sensitive cells enhanced attachment to laminin. Tumor cell attachment to, and detachment from, the sublaminal matrix protein laminin and type IV collagen are thought to be important events in the metastatic process. Our results indicate that tumor cell attachment to these proteins may be partially modulated by the expression of specific oligosaccharide structures associated with the cell surface.

Cell adhesion to extracellular matrix (ECM) is necessary for most nonlymphoid cells to proliferate and differentiate (1, 2). Many of the isolated structural components of ECM have been shown to mediate cell attachment in vitro, and these include collagen types I–V, fibronectin, laminin, chondronectin, entactin, and proteoglycans (1). The composition of ECMs depends on the type of cell producing the matrix. For example, fibroblasts synthesize and preferentially attach to fibronectin and types I and III collagen; the endothelial cell ECM that lines blood vessel walls contains laminin and type IV collagen (1). The large number of adhesion-mediating components in ECM, and the fact that some components have multiple binding sites has complicated the analysis of cell–substratum adhesion (1, 3). For example, fibronectin has binding sites for collagen, fibrin, heparin, polyamines, and the cell surface. Laminin binds to type IV collagen, heparin sulfate, and a cell surface receptor. To date the only cell surface receptor for attachment to vascular ECM to be isolated is that of laminin (4, 5). The laminin receptor may be particularly important to the metastatic phenotype because the ability of tumor cells to attach to laminin plus type IV collagen-coated surfaces in vitro has been shown to correlate with the lung-colonizing potential of the cells when they are administered intravenously (6).

Before attachment to ECM and invasion at a secondary site, the metastatic tumor cell escapes from the primary site and passes through the vascular ECM to enter the circulation. This process may be facilitated by the decrease in tumor cell adhesiveness to ECM proteins that often accompanies malignant transformation. Malignant cells often have reduced cy-

Abbreviations used in this paper: Asn, asparagine; Con A, concanavalin A; ECM, extracellular matrix; Gal, galactose; GlcNAc, N-acetylgalactosamine; IMDM, Iscove's modified Dulbecco's medium; Man, mannose; WGA*, wheat germ agglutinin-resistant; WGA*, wheat germ agglutinin-sensitive.
toskeletal organization and focal contacts, which may partially explain their less adhesive properties (7). It has also been suggested that the commonly observed increase in sialylation and consequently metastasis (8, 9). Recent studies on a number of mutant cell lines have provided evidence that changes in cell surface carbohydrates can affect cell attachment to isolated ECM proteins. Ricin-resistant baby hamster kidney cells (10) and wheat germ agglutinin-resistant (WGA') murine tumor cells (8) having lesions in the carbohydrate structure of their glycoconjugates also showed changes in adhesion to fibronectin and collagen. AD-6, a mutant of 3T3 fibroblasts which was selected for increased detachability from plastic surfaces, was subsequently found to have a defect in oligosaccharide biosynthesis (11). Adhesion of melanoma cells (12) and fibroblasts (13) to fibronectin-coated plastic is reduced by treating the cells with tunicamycin, an inhibitor of Asn-linked oligosaccharide synthesis.

There are a number of ways in which altered oligosaccharide structure may affect the normal function of glycoproteins involved in cell attachment. For example, inhibition of Asn-linked oligosaccharide synthesis by tunicamycin has been shown to reduce the activity of the insulin receptor (14), alter the conformation and inhibit cell surface expression of the G protein in vesicular stomatitis virus-infected cells (15), and to inhibit Fc-related functions of IgG2b monoclonal antibodies without changing the hapten specific binding activity (16). In addition, the oligosaccharide portion of the glycoprotein can mediate a biological function by binding to a lectin-like receptor, an example of this being the delivery of lysosomal enzymes to lysosomes via the mannose-6-P binding protein in the endoplasmic reticulum of mammalian cells (17).

Attachment of cells by membrane oligosaccharides to surfaces coated with lectins (18) glycosyltransferases (19) and glycosidases (18) has been shown to induce cell spreading similar to cells plated on ECM- or fibronectin-coated surfaces. Therefore, we have examined the possibility that some ECM molecules have lectin-like activities capable of binding cell surface oligosaccharides, thereby mediating attachment. Asn-linked glycopeptides have been isolated from MDW4, a WGA' mutant and from the metastatic parental tumor line MDA-D2 (20). The WGA' mutant attached more avidly to fibronectin- and type IV collagen-coated surfaces (8) and was shown to have a premature truncation of the antennae on the poly-N-acetyllactosamine-containing oligosaccharides found in the wild-type cells (20). Glycopeptides from mutant and wild-type cells as well as from standard glycoproteins were used in an attempt to inhibit MDW4 cell attachment to laminin, fibronectin, and type IV collagen, the major glycoprotein components of vascular ECM encountered by metastatic tumor cells. The results indicate that type IV collagen may bind to high mannos (Man) structures on the cell surface, and that a poly-N-acetyllactosamine-containing Asn-linked oligosaccharide found on the metastatic wheat germ agglutinin-sensitive (WGA') cells inhibits cell attachment to laminin. Tumor cell detachment from vascular ECM at the primary tumor sites and subsequent attachment to a structurally similar ECM at secondary sites may be facilitated by modulation of specific cell surface oligosaccharide structures.

**MATERIALS AND METHODS**

**Materials:** Type IV collagen and laminin were purified from the murine EHS sarcoma and kindly provided by R. Timpl (Max-Planck-Institut für Bio-chemie, München, Federal Republic of Germany) (21, 22). Serum fibronectin was purchased from Bethesda Research Laboratories (Gaithersburg, MD). Human transferrin, conalbumin type I, essentially fatty acid-free, fraction V BSA, and almond emulsion β-glucosidase type II were obtained from Sigma Chemical Co. (St. Louis, MO). Bio-Gel P-2 and P-6 came from Bio-Rad Laboratories (Mississauga, Ontario, Canada); DE52 cellulose from Whatman Chemical Separation, Inc. (Clifton, N.J.); concanavalin A-Sepharose-4B, DEAE Sephadex A-50, and Sephadex G25 from Pharmacia Fine Chemicals (Piscataway, N.J.; Quebec, Canada); and Reacti Gel (25DF) from Pierce Chemical Co. (Rockford, IL). Terasaki Microtest plates were purchased from Falcon Plastics (Canlab, Toronto, Ontario, Canada).

**Tumor Cells:** The origin and properties of the highly metastatic DBA/2 strain tumor called MDA-D2 has been described in detail (23, 24). The WGA' mutant MDW4 was isolated from a mu'tagenized population of MDA-D2 cells by selection in growth medium containing WGA' as previously described (25, 26). MDW4 cells have lost the phenotypic revertant property from a metastasis found in DBA/2 mouse which had been injected subcutaneously with MDW4 cells (25). The nonmetastatic MDW4 cells spontaneously hybridize in situ with a bone marrow-derived host cell thereby regaining the "wild type" (i.e., MDA-D2) lectin-resistant and the metastatic phenotypes (26).

Tumor cells were grown in suspension in RPMI-1640 tissue culture medium supplemented with 10% fetal calf serum, 100 U/ml penicillin, 100 μg/ml streptomycin, and 5 mM glutamine, (Grand Island Biological Co., Grand Islands, NY). Tumor cells used for attachment experiments were cultured 3-10 d before the experiment in Iscove's modified Dulbecco's medium (IMDM) supplemented with 5 μg/ml human transferrin, 1.5 μM ethanolamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 0.1 mg/ml essentially lipid-free BSA which had been detoxified on charcoal. The tumor cells divided at near normal rates and could be passaged in the secondary sites for extended periods of time (i.e., >1 mo). The cells were tested for mycoplasma by staining DNA with 4',6'-diamino-2-phenylindole and found to be free of contamination during the course of these experiments.

For lectin sensitivity tests, the cells at 5 x 10⁶/ml in fetal calf serum supplemented medium were plated in Limbro wells with concentrations of lectin ranging from 0 to 100 μg/ml. After 5 d the cells grown in absence of lectin had reached maximal density and the cell number in each concentration of lectin was determined.

**Glycopeptides:** The total cellular glycopeptides isolated from ~5 x 10⁶ tumor cells of each MDA-D2 and MDW4 were characterized as documented in reference 20 and used in the present study. Briefly, lipids were removed by extraction with chloroform/methanol (2:1) (vol/vol) and the residue was exhaustively digested in 1% (w/v) pronase followed by precipitation of glycosaminoglycans, DNA, and RNA by the addition of cetylpyridinium chloride to 4 mM. The glycopeptides were desalted on a Bio-Gel P-6 column then separated on Whatman DE52 cellulose column to obtain neutral and sialylated species. Each fraction separated on DE52 cellulose was desalted and applied to a concanavalin A-Sepharose-4B (Con A-Sepharose) column. The neutral and Con A-bound glycopeptides from WGA' and WGA' cells were shown to be similar by proton nuclear magnetic resonance analysis. These glycopeptides isolated during the study documented in reference 20 appeared by proton nuclear magnetic resonance analysis to be a mixture of ~40% Manα4Manα, 15% Manα, 15% Manα, 25% Manα, and 15% Manα, (structure 5 in Fig. 1) (20).

Complex glycopeptides are defined according to the sugar residues at the nonreducing ends, the Manα1-6 arm being named first; S denotes sialic acid, G is galactose (Gal), Gn is N-acetylgalactosamine (GlcNAc), and M is Mano. There was essentially no neutral glycopeptides in MDAY-D2 excluded by the Con A-Sepharose column but ~25% of the MDW4 glycopeptides were separated in this way and shown to be triantennary complex terminating in N-acetylglucosamine (GnGnGn, structure 3 in Fig. 1). The sialylated poly-N-acetyllactosamine-containing glycopeptide from MDAY-D2 cells bound to DE52 cellulose and was eluted in a symmetrically shaped peak. The total cellular glycopeptides isolated from ~5 x 10⁶ tumor cells of each MDA-D2 and MDW4 were characterized as documented in reference 20 and used in the present study. Briefly, lipids were removed by extraction with chloroform/methanol (2:1) (vol/vol) and the residue was exhaustively digested in 1% (w/v) pronase followed by precipitation of glycosaminoglycans, DNA, and RNA by the addition of cetylpyridinium chloride to 4 mM. The glycopeptides were desalted on a Bio-Gel P-6 column then separated on Whatman DE52 cellulose column to obtain neutral and sialylated species. Each fraction separated on DE52 cellulose was desalted and applied to a concanavalin A-Sepharose-4B (Con A-Sepharose) column. The neutral and Con A-bound glycopeptides from WGA' and WGA' cells were shown to be similar by proton nuclear magnetic resonance analysis.

**Structures:** Glycopeptides from 1 g of conalbumin and 1 g of human transferrin were isolated as follows. The glycoprotein was dissolved in 10 ml of 0.1 M Tris, pH 7.9, 1 M CaCl₂, 0.01 mM NaN₃, 100 μM promase, and 5 drops of toluene blue and incubated at 37°C for 3 d. After correcting the pH to 7.9 with 1 M HCl, 50 mg of pronase was added on days 2 and 3. The sample was applied to a Sepharose G25 column (2.5 x 90 cm) and eluted with water. The pronase digestion and desalting were repeated. The conalbumin glycopeptide is a bisected triantennary complex terminating in GlcNAc as shown in structure 4 in Fig. 1 (20).

It was retained by the G25 column and eluted in a broad symmetrical peak. The human transferrin glycopeptide has previously been shown to be mostly sialylated biantennary SAα2-6Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAcβ1-Asn (i.e., SS)
Figure 1 Structures of the Asn-linked glycopeptides tested as inhibitors of cell attachment in Fig. 3. Structure 1 (SSS) is the sialylated poly-N-acetyllactosamine-containing glycopeptide isolated from MDAY-D2 and is also present in the WGA i MDW4 versus host cell hybrid lines removed from a metastasis of an MDW4 injected mouse (e.g., MDW4-24a). Structure 2 is the asialo form of glycopeptide 1 (GGG) (20). Structure 3 is the mutant complex glycopeptide isolated from MDW4 cells (GnGnGn). Structure 4 is the conalbumin glycopeptide (GnGnGnGn) (27). Structure 5 represents the high-mannose glycopeptide (Man₅₋₉) isolated from both MDAY-D2 and MDW4 cells. Assuming that only the postulated intermediates of high-mannose processing were present (50), analysis of the proton nuclear magnetic resonance spectra indicated that the sample contained ~40% Man₁₀, 15% Man₉, 15% Man₈, 25% Man₇, and 15% Man₆. Man₅ to Man₉ are the structures resulting from the sequential removal of mannoside residues numbered 1–4. Structure 6 is the core glycopeptide MM isolated from human myeloma IgG (51). Structure 7 is Man₅ isolated from ovalbumin (52). R represents GlcNAcβ1–4GlcNAcβ1–Asn.

RESULTS

Tumor Cell Attachment

In an earlier study, tumor cells were grown in RPMI-1640 supplemented with fetal calf serum and transferred to serum-free medium for use in the attachment assay (8). Serum contains fibronectin as well as other attachment factors that may bind to the cells and interfere with the attachment assays. Therefore a serum-free medium that can support long-term growth of the MDAY-D2 cells and MDAY-D2 variants was developed. The Iscove's-based serum-free medium supplemented with transferrin, ethanolamine, and BSA as described in Materials and Methods supported cell growth for months in tissue culture at a rate similar to that of serum-containing medium. Tumor cells grown in serum-free medium attached to laminin, fibronectin, and type IV collagen in the same manner as cells from serum with one exception. WGA i (i.e., MDAY-D2 and MDW4-24a) cells from serum-containing medium attached to laminin (8), but the same cells grew d in serum-free medium did not attach to laminin. The results in Fig. 2 show that for cells grown in serum-free medium, the nonmetastatic WGA i mutant (MDW4) was more adhesive than the WGA i MDW4-24a cells on fibronectin, laminin, and type IV collagen. The parental line MDAY-D2 had an attachment phenotype identical to that of the MDW4 versus host cell hybrid (i.e., MDW4-24a) (8). Because MDW4 and MDW4-24a were near tetraploid lines and therefore larger cells than MDAY-D2, they were compared in the experiments on inhibition of attachment described here.

MDW4 has previously been shown to have three to four times less neuraminidase-releasable cell surface sialic acid compared with MDAY-D2 and the MDW4 versus host cell hybrid cells and, in addition, the removal of sialic acid from WGA i cells with neuraminidase enhanced cell attachment to type IV collagen and fibronectin (8). These results led to the suggestion that an asialo-glycoconjugate may be involved in MDW4 adhesion. A more detailed examination of the Asn-linked oligosaccharides in mutant and WGA i cells indicated that complex oligosaccharides in the mutant were also deficient in Gal and terminated in GlcNac (Fig. 1) (20). The mutant was also resistant to the Gal-binding lectin leucoagglutinin (32) and hypersensitive to the GlcNAc-binding lectin from Bandeiraea simplicifolia seed (33) in agreement with
the observed changes in oligosaccharide structure and the specificities of these lectins (Table I). In an earlier study on lectin binding, MDW4 was found to have an increase in cell surface Con A-binding sites (34), which probably accounts for the increased sensitivity of MDW4 cells to Con A in tissue culture (Table I).

To summarize, the WGA\(^{\text{a}}\) cells (i.e., MDAY-D2 and MDW4 versus host cell hybrids) had sialylated poly-N-acetyl-lactosamine-containing complex (structure 1 in Fig. 1) which has been replaced in the mutant by GlcNAc terminating complex consisting of a mixture of \(\sim 33\%\) triantennary with GlcNAc 2, 4 on the Man\(\alpha 1-3\) and 66\% triantennary with the GlcNAc 2, 6 on the Man\(\alpha 1-6\) (structure 3 in Fig. 1) (20). The mutant also has an apparent increase in Con A-binding oligosaccharides on the cell surface, possibly of the high mannose type (structure 5 in Fig. 1). The GlcNAc terminating complex isolated from the mutant as well as the Con A-binding high mannose-type glycopeptides appeared to be reasonable candidates for oligosaccharides that might be involved in the increase adhesion of MDW4 cells to substratum.

**Glycopeptide Inhibition of MDW4 Cell Attachment**

In experiments testing the glycopeptide inhibition of cell attachment, microtest wells were coated with a concentration of substrate determined on the same day to give 50–70\% cell attachment. The glycopeptides were added at a concentration of 0.2 nmol/10-\(\mu\)l well (i.e., 20 \(\mu\)M), approximately three orders of magnitude more than the glycopeptide concentration present in the 500 cells added to each well. The results in Fig. 3 show that SSS inhibited MDW4 cell attachment to laminin but not to fibronectin or type IV collagen. GGG, the same glycopeptide with sialic acid removed also inhibited attachment but the mutant glycopeptide G\(\text{GGG}\) showed no activity. Glycopeptides with the same terminal residues as those on the inhibitory structures, namely S\(\text{S}\) and G\(\text{G}\) from human transferrin did not inhibit cell attachment to laminin (Fig. 4). A titration of the inhibitors in Fig. 4 showed that the concentration of SSS and GGG required for 50\% inhibition

![Figure 2](image_url)

**FIGURE 2** Tumor cell attachment (performed as described in Materials and Methods) as a function of increasing substrate concentration. \(\Delta\), attachment of the WGA\(^{\text{a}}\) mutant MDW4; \(\bullet\), attachment of a WGA\(^{\text{a}}\) MDW4 versus host cell hybrid removed from a metastasis of an MDW4-injected mouse (i.e., MDW4-24a) to laminin, fibronectin, and type IV collagen was performed as described in Materials and Methods.

![Graph](graph_url)

**TABLE I**

| Cell line  | WGA  | PHA(1) | BS\(_{\text{u}}\) | Con A | Metas- | Substr- |
|------------|------|--------|----------------|-------|tasis\(^{\text{a}}\) | tatum adhesion\(^{\text{a}}\) |
| MDAY-D2    | 7    | 7      | >100           | 33    | high  | low    |
| MDW4       | >50  | >50    | 6              | 14    | non   | high   |
| MDW4-24a   | 7    | 6      | >100           | 40    | high  | low    |

Abbreviations: BS\(_{\text{u}}\), Bandeirea simplifolia lectin II; PHA(1), leucoagglutinin lectin.

\(^{a}\) The lectin sensitivities were determined as described in Material and Methods. The concentration of lectin reducing the cell number to 10\% of the control after 5 d of tissue culture is the D10 value.

\(^{a}\) MDW4 cells grew at the subcutaneous site of injection and only the progeny of hybrids between MDW4 and a bone marrow-derived host cell were found in the metastases (e.g., MDW4-24a). The hybrids had regained both the lectin resistance profile and the high metastatic capacity of the parental line MDAY-D2 (23, 26).

\(^{a}\) MDW4 cells attach more avidly than MDAY-D2 and MDW4-24a to fibronectin, laminin, and type IV collagen in vitro.
FIGURE 3 Inhibition of MDW4 cell attachment to fibronectin, laminin, and type IV collagen by the Asn-linked glycopeptide shown in Fig. 2. Microtest wells were coated with 5 μg of BSA and either 7.8 ng of laminin, 3.9 ng fibronectin, or 3.9 ng type IV collagen and glycopeptides were added at a concentration of 20 μM. Attachment of MDW4 cells to the respective substrates in the presence and absence of glycopeptides was used to calculate inhibition. The results presented with standard error bars are the mean inhibition values of three to six experiments performed in triplicate, the others are the mean of two experiments performed in triplicate.

FIGURE 4 Inhibition of MDW4 cell attachment to laminin as a function of glycopeptide concentration. Microtest plate wells were coated with 15.6 ng of laminin plus 5 μg of BSA. The glycopeptides used were human transferrin GG (△); human transferrin SS (○); wild-type cellular CCG (●); and wild-type cellular SSS (□). Each point is the mean of three replicates and error bars represent the standard error.

was ~1.5 and 0.3 M, respectively. SSS did not appear to inhibit attachment by binding avidly to laminin as preincubation of laminin on the microtest plates with SSS then washing did not inhibit MDW4 cell attachment (data not shown).

MM, Man₅, and the mixture Man₅₋₉ inhibited MDW4 cell attachment to type IV collagen but not to fibronectin (Fig. 3). Half-maximal inhibition of cell attachment for MM and Man₅ was observed at 0.3 and 1 μM, respectively, and the mixture Man₅₋₉ was less active with 50% inhibition at ~10 μM (Fig. 5). This would be expected if the Man₅ component of Man₅₋₉ (i.e., 15%) were the major inhibitor in the mixture. None of the complex structures, 1, 2, 3, or 4 shown in Fig. 1 inhibited MDW4 attachment to type IV collagen (Fig. 3).

Attachment of Peptide N-Glycosidase-treated Cells

Asn-linked oligosaccharides on the intact cell surface have been shown to be susceptible to cleavage by peptide N-glycosidase (35). The enzyme releases intact complex and high mannose oligosaccharides from the protein leaving the ε-amino of the Asn on the reducing end. Both MDW4 and MDW4-24a cells were treated with peptide N-glycosidase and compared to mock-treated cells for attachment to type IV collagen and laminin (Fig. 6). Enzyme-treated MDW4 cells were less adhesive on type IV collagen as would be expected if the mechanism of type IV collagen binding required the recognition of cell surface high mannose structures. There appears to be less high Man structures on the WGA⁺ cells (34) and this may account for their nonadhesive behavior on type IV collagen (Fig. 2). As would be predicted from this line of reasoning, MDW4-24a attachment to type IV collagen was not affected by peptide N-glycosidase treatment.

Although treatment of MDW4 cells inhibited attachment to type IV collagen, no effect was seen for attachment to laminin suggesting that Asn-linked oligosaccharides were not mediating cell attachment to laminin. MDW4-24a cells became more adhesive to laminin after peptide N-glycosidase treatment (Fig. 6). This result is consistent with the idea that WGA⁺ cells can attach to laminin provided that an inhibiting Asn-linked oligosaccharide is removed, presumably the SSS structure. The enzyme treatment did not restore the attachment phenotype of MDW4-24a on laminin to that of MDW4 possibly due to the incomplete removal of SSS from the cell surface (Fig. 6, inset). The removal of the oligosaccharide...
from native glycoproteins by peptide N-glycosidase has been shown to vary depending on tertiary structure and accessibility of the oligosaccharides (30).

**DISCUSSION**

Attachment studies using lectin-resistant mutants with lesions in oligosaccharide synthesis (8, 10) and the use of the inhibitor tunicamycin (12, 13) have provided indirect evidence that Asn-linked oligosaccharides are required for various types of cell adhesion phenomenon. In this report, Asn-linked glycopeptides isolated from a WGA" mutant adhesive on fibronectin, laminin, and type IV collagen, and from the nonattaching wild-type cells were tested for their ability to inhibit cell attachment.

MDW4 cell attachment to type IV collagen was inhibited by the mannose terminating glycopeptides MM and Man5 and to a lesser degree by the mixture Man5-9. MDW4 cells have more cell surface Con A-binding sites than the WGA" cells (34). Because the major Con A-binding glycopeptides isolated from these cells were the high mannose structures (20), it is possible that inhibition of MDW4 attachment to type IV collagen by the Man terminating glycopeptides was due to competition with similar structures on the cell surface for an oligosaccharide-binding site in the collagen. Removal of Asn-linked oligosaccharides by almund emulsin peptide N-glycosidase treatment reduced MDW4 attachment to type IV collagen supporting the hypothesis that Asn-linked oligosaccharides (e.g., Man5) may directly mediate cell attachment to type IV collagen. Man6-9 species are usually found inside the cell as processing intermediates in the production of complex, Man5, and hybrid structures (36). Therefore, the finding that Man5 glycopeptide was a more potent inhibitor than the mixture Man5-9 may be due to a preference of type IV collagen for the processed high mannose structure. A lectin activity on teratocarcinoma cells recognizing high mannose oligosaccharides has been reported to mediate homotypic cell adhesion (37). Because laminin and type IV collagen have been isolated from teratocarcinoma ECM (38), it is possible that the carbohydrate binding component detected in this study was type IV collagen.

In that the major change in Asn-linked oligosaccharides in the mutant cells was the acquisition of GnGnGn (20), it was suspected that this structure may be important for the increased adhesion of the cells to one or more ECM proteins. However, the mutant GnGnGn structure did not inhibit attachment to laminin, fibronectin, or type IV collagen at the concentrations tested. This does not eliminate the possibility that the presence of GnGnGn or absence of the wild-type structure affects the expression or activity of a glycoprotein involved in cell attachment. Rather than the presence of a mutant oligosaccharide, the absence of the wild type SSS appeared to be the cause of increased MDW4 attachment to laminin. SSS is absent from MDW4 cells yet its addition to the attachment assay inhibited MDW4 cell attachment to laminin, possibly due to steric interference at the laminin receptor-laminin binding site. Laminin receptors on MDW4-24a cells may be glycosylated with the SSS structure consequently blocking laminin binding or the proximity of SSS on other cell surface glycoproteins may be sufficient to block cell attachment to laminin. MDW4-24a cells showed increased attachment to laminin after they had been treated with peptide N-glycosidase presumably due to the partial removal of SSS from the cell surface.

A number of other structurally related glycopeptides did not inhibit cell attachment to laminin. SS, GG, and the triantennary GnGnGn were not inhibitory, indicating that recognition of SSS and GGG required more of the structure than terminal monosaccharides, and that triantennary substitution of the core was also insufficient. It is possible that the repeating Galβ1-4GlcNAc in the antenna of SSS was functionally important for inhibiting cell attachment to laminin. Mobile cells encountering laminin in vascular ECM such as erythrocytes (39) and the K562 leukemia (40) have been shown to have the large Asn-linked poly-N-acetyllactosamine-containing glycopeptide. In addition, embryonal carcinoma cells appear to have a similar structure that disappears during in vitro differentiation (41). Because laminin appears at an early stage in embryogenesis (42), the presence of the poly-N-acetyllactosamine-containing oligosaccharide could play a
role in embryonic cell detachment and movement.

MDW4 cell attachment to fibronectin was not inhibited by the Asn-linked glycopeptides used in this study. It has been suggested that ganglioside GTIb mediates cell attachment to fibronectin (43); however, GTIb also inhibited cell attachment to gelatin and lectins indicating a lack of specificity (18). Lesions in the glucosaminoglycans, glycolipids, and O-linked oligosaccharides of MDW4 are being investigated as possible candidates for the increased adhesion of MDW4 to fibronectin.

The cellular environment can have a profound effect both qualitatively and quantitatively on the cell surface oligosaccharide structures. Confluent cells compared with cells in log-phase growth have increased high mannose to complex ratios (44). A recent study comparing tumor cells growing at a subcutaneous site with tumor cells in the liver demonstrated changes in the WGA-binding glycoproteins of the plasma membrane due apparently to the tissue environment since transplanting the tumor cells from the subcutaneous site to liver or the reverse was accompanied by a rapid reverse in the glycoprotein pattern (45). Therefore, differences in tissue environments which influence cell surface oligosaccharides expression may allow metastatic tumor cells to become relatively nonadherent to ECM proteins at the primary and, subsequently, attach at the secondary site to similar ECM proteins. Modulation of adhesion by altering oligosaccharide structure has been observed in embryonic tissue. Removal of sialic acid from oligosaccharides on retinal cell–cell adhesion molecules accompanies development from embryonic to adult forms and from inactive to active homotypic binding (45).

Yogeeswaran and Salk (9) have shown that the metastatic capacity of a range of murine tumor cell lines is positively correlated with the degree of sialylation of available galactose and N-acetylgalactosamine residues on the cell surface glycoconjugates. These results suggest that the more completed (i.e., sialylated) the complex oligosaccharides, the more metastatic and autonomous from host growth controls the tumor cell becomes. A number of under-sialylated WGA mutants isolated from the B16 melanoma (47), RAW-117-H10 lymphosarcoma (48) and MDAY-D2 (25) have been found to be less metastatic than the respective parental cell line. For example, MDW4 has been shown to be more susceptible to MDAY-D2 and to MDAY-D2-like tumor cells in the liver demonstrated changes in the WGA-binding glycoproteins of the plasma membrane due apparently to the tissue environment since transplanting the tumor cells from the subcutaneous site to liver or the reverse was accompanied by a rapid reverse in the glycoprotein pattern (45). Therefore, differences in tissue environments which influence cell surface oligosaccharides expression may allow metastatic tumor cells to become relatively nonadherent to ECM proteins at the primary and, subsequently, attach at the secondary site to similar ECM proteins. Modulation of adhesion by altering oligosaccharide structure has been observed in embryonic tissue. Removal of sialic acid from oligosaccharides on retinal cell–cell adhesion molecules accompanies development from embryonic to adult forms and from inactive to active homotypic binding (45).

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