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Reduced Contact-Inhibition and Substratum Adhesion in Epithelial Cells Expressing GlcNAc-Transferase V

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Abstract. Malignant transformation of fibroblast and epithelial cells is accompanied by increased β1–6 *N*-acetylglucosaminyltransferase V (GlcNAc-TV) activity, a Golgi N-linked oligosaccharide processing enzyme. Herein, we report that expression of GlcNAc-TV in Mv1Lu cells, an immortalized lung epithelial cell line results in loss of contact-inhibition of cell growth, an effect that was blocked by swainsonine, an inhibitor of Golgi processing enzyme α-mannosidase II. In serum-deprived and high density monolayer cultures, the GlcNAc-TV transfectants formed foci, maintained microfilaments characteristic of proliferating cells, and also experienced accelerated cell death by apoptosis. Injection of the GlcNAc-TV transfectants into nude mice produced a 50% incidence of benign tumors, and progressively growing tumors in 2:12 mice with a latency of 6 mo, while no growth was observed in mice injected with control cells. In short term adhesion assays, the GlcNAc-TV expressing cells were less adhesive on surfaces coated with fibronectin and collagen type IV, but no changes were observed in levels of cell surface $\alpha_5\beta_1$ or $\alpha_v\beta_3$ integrins. The larger apparent molecular weights of the LAMP-2 glycoprotein and integrin glycoproteins α_5, α_v and β_1 in the transfected cells indicates that their oligosaccharide chains are substrates for GlcNAc-TV. The results suggest that β1–6GlcNAc branching of N-linked oligosaccharides contributes directly to relaxed growth controls and reduce substratum adhesion in premalignant epithelial cells.

N-LINKED glycosylation of proteins begins in the lumen of the rough endoplasmic reticulum where a subset of Asn-X-Ser/Thr residues on newly synthesized proteins are subjected to substitution with $Glc_3Man_9GlcNAc_2$. The oligosaccharides are then remodeled or processed as the newly synthesized glycoproteins are transported through the Golgi compartments en route to the cell surface (Kornfeld and Kornfeld, 1985; Schachter, 1986). Many of the glycosyltransferases and glycosidases that constitute the Golgi oligosaccharide processing pathways are regulated in a tissue-specific manner (Paulson et al., 1989). Their patterns of expression and acceptor specificity appear to be the basis for the plethora of oligosaccharide structures observed in secreted and membrane glycoproteins. For the complex-type N-linked oligosaccharides, structural diversity begins with GlcNAc-branching of the trimannosyl core and continues with a variety of glycosyltransferases that complete these antennae. The β1–6GlcNAc-linked antenna which is initiated by the action of β1–6 *N*-acetylglucosaminyltransferase V (GlcNAc-TV)[1] is the preferred substrate for β1-3GlcNAc-T(i), a rate-limiting enzyme in the pathway leading to polylactosamine (i.e., repeating Galβ1-4GlcNAcβ1-3), extended-chain Lewis antigens and blood-group sequences (van den Eijnden et al., 1988; Yousefi et al., 1991; Heffernan et al., 1993). In most human epithelial tissues, expression of β1–6GlcNAc-branching as well as Lewis and blood-group antigens is low, but after transformation expression increased, and as such, these oligosaccharides are significant markers of carcinoma (Kim et al., 1986; Itzkowitz et al., 1986; Hakomori, 1989). GlcNAc-TV activity is increased 2–10-fold in rodent fibroblast lines transfected with activated oncogenes in the *ras*-signaling pathway (i.e., T24 *H-ras*, *v-src*, *v-fps*, middle T of polyoma virus) (Yamashita et al., 1985; Dennis et al., 1987, 1989; Lu and Chaney, 1993; Palcic et al., 1990).

Somatic mutations (Dennis, 1986; Dennis et al., 1987) and chemical inhibitors of N-linked oligosaccharide processing that block expression of β1–6GlcNAc branched glycans can reverse some features of the transformed phenotype. For example, castanospermine and deoxynorjiromycin, inhibitors of Golgi α-glucosidase I have been shown to reverse the transformed morphology of fibroblasts expressing *v-sea* (Knight et al., 1988) and *v-fms* oncogenes (Nichols et al., 1985; Hadwiger et al., 1986). Swainsonine, a potent competitive inhibitor of Golgi α-mannosidase II blocks both tumor cell and trophoblast cell in-

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1. *Abbreviations used in this paper*: GlcNAc-TV, β1–6 *N*-acetylglucosaminyltransferase V; LAMP, lysosomal-associated membrane glycoprotein.

vasion through extracellular matrix, and increases cell-substratum adhesion in vitro (Yagel et al., 1989; Seftor et al., 1991). Swainsonine has also been shown to inhibit growth of T24 *H-ras*–transfected NIH 3T3 cells in soft agar (DeSantis et al., 1987), to reduce murine lymphoma and human carinoma cell proliferation in serum-free medium, and slow solid tumor growth in mice (Dennis et al., 1990; VanderElst and Dennis, 1991). The ability to inhibit features of cellular transformation with oligosaccharide processing inhibitors suggests certain cancer-associated changes in glycosylation may contribute directly to cellular transformation.

Furthermore, changes in glycosylation are known to occur early in tumor progression (Hakomori, 1989) and up-regulation of GlcNAc-TV may contribute to altered growth properties of premalignant cells. In this regard, the LEC strain of rats which have a hereditary predisposition to hepatitis and hepatocarcinomas show elevated levels of GlcNAc-TV mRNA in premalignant hepatitis, as well as in tumors and metastases (Miyoshi et al., 1993). To determine the role of β1–6GlcNAc branching in the early events of cellular transformation, we transfected Mv1Lu, an immortalized lung epithelial cell line with a GlcNAc-TV expression vector. The GlcNAc-TV expressing cells exhibited characteristics of cellular transformation, including loss of contact-inhibition, increased susceptibility to apoptosis, decreased substratum adhesion, and increased tumorigenicity in nude mice.

Materials and Methods

Cell Lines

Mv1Lu mink lung epithelial cells were cotransfected with pCD1rGNT-V and pSVneo using the calcium phosphate precipitate method, and after 2 d the cells were cultured in 600 μg/ml of G418 (GIBCO BRL, Gaithersburg, MD). The plasmids pCD1rGlcNAc-TV contained the rat GlcNAc-TV cDNAs driven by a CMV promoter, and was generously provided by Drs. Michael Pierce and N. Fregien (Shoreibah et al., 1993). Mv1Lu lung epithelial cells and transfectants were cultured in α-MEM supplemented with 10% FCS (Gibco).

To measure sensitivity to lectins, cells were inoculated at 2×10^4 cells/well into 24-well plates containing serial dilutions of leukoagglutinin (L-PHA) (Sigma Chem. Co., St. Louis, MO) beginning at 100 μg/ml. Sensitivity to lectin toxicity was determined by examining the cultures for cell growth 4 d later.

GlcNAc-TV Assay

Cells were washed in PBS and lysed in 0.9% NaCl, 1% Triton X-100 at 0°C. The reactions contained 16 μl of cell lysate, 0.5 μCi of [^{3}H]sugar-nucleotide donor (~16,000 dpm/nmol) in a total volume of 33 μl and were incubated for 2 h at 37°C (Yousefi et al., 1991). Endogenous activity was measured in the absence of acceptor, and subtracted from values determined in the presence of added acceptor. The β1–6GlcNAc-TV reactions contained 50 mM MES, pH 6.5, 1 mM UDP-[6-^{3}H]*N*-acetyl-glucosamine (26.8 Ci/mmol, Du Pont-New England Nuclear, Boston, MA), 0.1 M GlcNAc, 1 mM of GlcNAcβ1-2Manβ1–6Manα1-O$(CH_2)_8COOCH_3$. The reactions were diluted to 5 ml in H_2O and applied to a C18 Sep-Pak (Millipore Waters, Milford, MA) in H_2O, washed with 20 ml H_2O. The products were then eluted with 5 ml of methanol and radioactivity was counted in a β-liquid scintillation counter.

Repair of Wounded Cell Monolayers

Confluent-monolayers of cells were maintained in serum-containing medium for at least 6 d, and then in serum-free α-MEM medium for 24 h followed by denuding a lane with a plastic pipette tip. The cells were cultured in serum-free medium for an additional 24 h and then fixed with 1.5% paraformaldhyde in PBS, stained with 0.06% methylene blue and photographed under an inverted microscope. In swainsonine-treated cultures, 1 μg/ml of drug was present continuously from the initiation of the cultures. TGFβ1-treated cultures were supplemented with 50 pM cytokine when the cultures were wounded.

Cell Viability and Apoptosis

Cells were harvested with trypsin/EDTA in PBS, and an aliquot was diluted 1:5 with 0.4% trypan blue and then viable cells were counted in triplicate with a haemacytometer. Low molecular weight DNA was extracted from the remainder of the sample using the Hirts method (Hirt, 1967), separated by electrophoresis in 1.5% agarose gels and DNA was visualized by ethidium bromide staining.

Cell Attachment Assay

Cells in log phase growth were harvested by trypsin/EDTA, washed two times in PBS and resuspended in α-MEM at 10^6 cells/ml. The cells were applied in 100-μl aliquots to 96-well plates coated with serial dilutions of fibronectin and collagen type IV. Plates were precoated with 50 μl of the proteins diluted in water and air-dried overnight followed by blocking with 2.5 mg/ml of BSA in α-MEM at 37°C for 1 h. The cells were allowed to attach at 37°C for 30 min on fibronectin and 90 min on collagen type IV followed by washing three times with 200 μl of PBS. The attached cells were fixed and stained in 1% glutaraldehyde and 0.2% methylene blue for 4–16 h. The plates were washed in water and stained cells were quantified by adding 100 μl of methanol:acetic acid: water 4:1:4 and measuring A_{570} on a plate reader.

Microfilament Staining

Cells were fixed with 1% paraformaldyhyde, 0.2% glutaraldyhyde in PBS for 5 min at 0°C, and then solubilized with 0.5% Triton X-100, 50 mM NaCl, 30 mM sucrose, 10 mM Tris, pH 7.5, washed twice with PBS and incubated with 250 ng/ml of TRITC-labeled phalloidin (Sigma) for 30 min at 20°C. After washing twice with PBS, the cells were examined under monochromatic light and photographed using a Leitz DMRXE microscope.

Integrin Immunoprecipitations

Cells from 80% confluent cultures were detached from plates with 1 mM EDTA in PBS and 10^6 cells in 0.5 ml of PBS were cell surface labeled with 0.5 mCi of $Na^{125}I$ using Iodogen (Pierce, Rockford, IL). Cells were lysed in PBS containing 0.1% SDS, Triton X-100, 0.5% sodium deoxycholate and 1 mM PMSF, and then immunoprecipitates were prepared with either anti-α_5 antiserum (P1D6), or anti-vitronectin receptor antibodies (VNR147) (Telios, San Diego, CA) as previously described (Dedhar et al., 1991). Immunoprecipitates were analyzed by electophoresis in 7.5% SDS-polyacrylamine gels followed by autoradiography.

Western and Lectin Blots

Cells were lysed in 1% Triton X-100, 150 mM NaCl, 20 mM Tris pH 8.0, 5 mM EDTA at 4°C for 30 min. Proteins separated by SDS-PAGE under reducing conditions were transferred electrophoretically onto PVDF membranes (ICN Biomedicals, Costa Mesa, CA) in buffer consisting of 25 mM Tris, 0.2 M glycine which contained 20% methanol. Western blots and immunoprecipitations of LAMP-2 were performed as previously described (Nabi et al., 1991; Nabi and Rodriguez-Boulan, 1993). For detection of L-PHA reactive oligosaccharides, LAMP-2 was immunoprecipitated from 250 μg of protein cell lysate and after SDS-PAGE, blots were probed with 0.1 μg/ml of L-PHA–coupled horseradish peroxidase (E-Y laboratory) (0.1 μg/ml) in PBS, 0.1% BSA, 0.1% Tween 20. After three washes blots were developed with the ECL Chemiluminescence system (Amersham, UK) according to manufacturer's instructions, and exposed to X-ray film for periods of 15 s–5 min. Protein concentrations were determined using the BCA reagent (Pierce) and BSA as the standard.

Results

GlcNAc-TV Transfectants of Mv1Lu Cells

Mv1Lu cells and C1, a vector-transfected control cell line

expressed low levels of GlcNAc-TV (i.e., 60 pmol/mg/h) similar to that previously reported for nonmalignant fibroblasts and epithelial cell lines (Yamashita et al., 1985; Dennis et al., 1987, 1989; Lu and Chaney, 1993; Palcic et al., 1990). Enzyme activity in the transfectants ranged from 2.5–18 times that measured in Mv1Lu cells and C1, levels similar to that reported in *H-ras* transformed cell lines (Yamashita et al., 1985; Dennis et al., 1987, 1989; Lu and Chaney, 1993; Palcic et al., 1990) (Table I). Leukoagglutin (L-PHA) lectin binds to β1–6GlcNAc-branched N-linked oligosaccharides (Cummings and Kornfeld, 1982), and as expected, sensitivity of the transfectants to L-PHA toxicity correlated with GlcNAc-TV activity (Table I).

Lysosomal-associated membrane glycoprotein (LAMPs) in transformed cell lines have previously been shown to carry β1–6GlcNAc-branched N-linked oligosaccharides (Dennis et al., 1987; Laferte and Dennis, 1989). LAMPs are widely distributed glycoproteins with 16–18 N-linked carbohydrate chains (Fukuda et al., 1988; Heffernan and Dennis, 1989), and therefore provide a convenient marker for assessing changes in glycosylation. As shown in Fig. 1, the apparent molecular weight of endogenous LAMP-2 in the transfectants correlated with GlcNAc-TV activity (i.e., Mv1Lu ≤ R2 < M9 < M1), which is consistent with the expected increase in mass due to the presence of SA-Galβ1-4GlcNAcβ1–6 linked antennae in complex-type oligosaccharides. LAMP-2 in M1 and M9 cells also showed increased L-PHA reactivity confirming the presence of β1–6 linked antennae, while for LAMP-2 in R2 cells, branching was below the level of detection by L-PHA blotting (Fig. 1).

Confluent cultures of Mv1Lu cells are monolayers of nonoverlapping cells, whereas the transfectants showed a more retractile morphology, characteristic of transformation as well as foci formation (Fig. 2, *A* and *B*). These features of morphologic transformation were most pronounced in M1 cultures and intermediate for M9, R9, and R2 cells. However, foci formation appeared to involve a minority of cells in the cultures. Therefore, to confirm that foci-formation was associated with ectopic expression of GlcNAc-TV rather than a secondary change in the cell population, additional transfectants were examined for foci formation and GlcNAc-TV expression at first passage after selection in G418. The first passage transfectants were seeded at low density to observe the growth of individual cell colonies. Cells from GlcNAc-TV expressing transfectants (e.g., Fig. 2 *C*, *lower left*) formed foci at the center of each colony, while G418-resistant clones with basal levels of enzyme remained flat. Confluent cultures of GlcNAc-TV expressing cells also shed more cellular debris into the medium which may be a characteristic associated with failure to arrest growth and apoptotic cell death as described below. Cell growth rates in medium containing 10% FCS was similar for the GlcNAc-TV expressing transfectants and control cell lines (data not shown).

Table I. Characterization of GlcNAc-TV Transfected Mv1Lu Cells

Cell line	GlcNAc-TV	L-PHA toxicity	Tumorigenicity*
	pmol/mg/h	D_{50} *μg/ml*	
Mv1Lu	63 ± 6	>100	0/4
C1	59 ± 5	>100	0/4
R2	151 ± 15	100	ND
R9	277 ± 95	50	1/4
M9	567 ± 265	20	2/4
M1	1082 ± 308	10	3/4

C1 is a pSV2neo-transfected Mv1Lu cell line, and R2, R9, M9, and M1 are independent GlcNAc-TV transfectants. L-PHA sensitivity and GlcNAc-TV activity was measured as described in Materials and Methods.

*Tumors in balb/c nude mice detected 3–6 wk after subcutaneous injection of 10^6 remained small and appeared to be benign. Two mice injected with M9 cells showed progressive tumor growth at the site of injection after 6 mo, while no tumors were observed in the control groups.

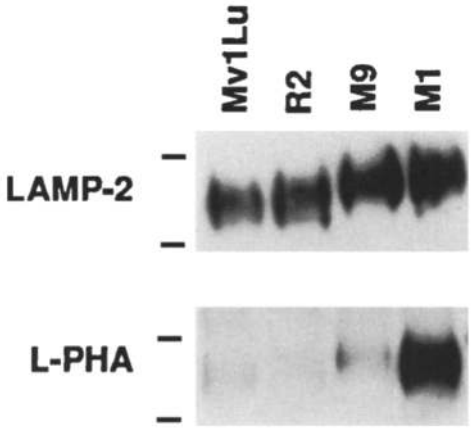

Figure 1. Western blot for detection of LAMP-2 glycoprotein (*top*). L-PHA lectin-blot of immunoprecipitated LAMP-2 for detection of β1–6GlcNAc-branched oligosaccharides (*bottom*). Horizontal lines are positions of 180 kD and 98 kD, respectively.

Subcutaneous injections of the transfectants into nude mice produced benign tumors at the site of injection in 6:12 mice, while incidence was 0:8 for Mv1Lu- and C1-injected mice (Table I). After 6–8 mo, tumors growing progressively were observed in two mice injected with M9 cells and no tumors were observed in control groups. This long latency period suggests that an additional genetic event or mutation occurs in the benign tumors, and is required to produce malignancies. This contrasts with introduction of activation *H-ras* into Mv1Lu cells which results in a highly tumorigenic phenotype, with rapidly growing tumors in all mice at 3–6 wk (Khan et al., 1991).

Release from Topoinhibition and Apoptosis in GlcNAc-TV Expressing Cells

Mv1Lu cells become growth-arrested in G0/G1 at high density and in the absence of growth factors (Ewen et al., 1993). Contact-inhibited cultures grown under these conditions were scratch wounded and examined for the rate of wound repair. Similar experiments have previously been used to study the relationship between contact- or topoinhibition and serum requirements in transformed and nontransformed cells (Delbecco, 1970). As shown in Fig. 3, the GlcNAc-TV transfectants migrated into a scratch wound more rapidly than Mv1Lu cells and C1, the pSV2neo transfected control (Fig. 3). The slower moving Mv1Lu cells maintained a tightly packed monolayer appearance at the edge of the wound, while the transfectants

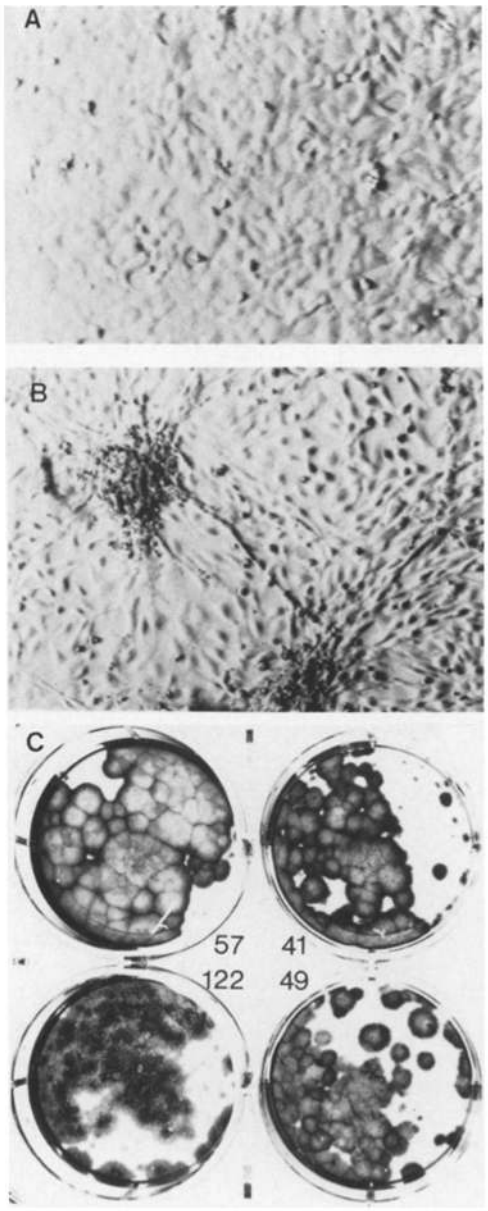

Figure 2. Morphology of (*A*) Mv1Lu cells and (*B*) the GlcNAc-TV transfectant M1 when cultures were maintained at confluence for 6 d in α-MEM plus 10% FCS. Photographed with 40× objective. (*C*) In a second cotransfection experiment, with pCD1rGNT-V and pSVneo plasmids, G418-resistant colonies were examined for altered morphology at first passage. Colonies were seeded into 3.4-cm wells at 50 cells/well and grown for 10 d in α-MEM plus 10% FCS. In parallel, the cells were expanded to 10^7 cells to assay GlcNAc-TV activity which is indicated by the figure beside each well in pmol/mg/h. The colony seeded into the lower left well expressed GlcNAc-TV, while the other three clones showed levels comparable to Mv1Lu cells.

moved into the wound leaving space between the advancing cells. The number of cells that migrated into the scratch wound after 24 h was 4–10 times greater for the transfectants than Mv1Lu or the C1 clone, and they occupied most of the surface area of the wound (Table II). The addition of serum-containing medium restored the ability of Mv1Lu cells to repair the wounded area by 24 h (data not shown). Therefore, expression of GlcNAc-TV markedly reduced the serum-growth requirement of contact-inhibited Mv1Lu cells.

Cultures treated with the processing inhibitor swainsonine which blocks expression of β1–6GlcNAc-branched oligosaccharides, reduced the rate of cell migration by the transfectants into the scratch wound by 50–70% (Fig. 4 *C*). This confirmed that expression of the GlcNAc-TV product is a requisite of this cellular phenotype. TGF-β has been shown to induce G1 growth arrest in Mv1Lu cells, and to prevent repair in the scratch wound assay (Ewen et al., 1993). The GlcNAc-TV transfectants remained responsive to the antiproliferative effects of TGF-β1 (Fig. 4 *D*). Furthermore, TGF-β1 blocked proliferation of the transfectants as determined by suppression of [^{3}H]thymidine incorporation into DNA (data not shown).

In tissue culture, motile and proliferating epithelial and fibroblast cells have prominent microfilament stress fibers and focal contacts, while in contact-inhibited cells, microfilaments are largely disassembled (Burridge et al., 1988). TRITC-phalloidin staining of microfilaments showed that stress fibers and focal adhesions were much more evident in the GlcNAc-TV transfectants than in Mv1Lu cells under the high density low serum conditions used in the scratch wound assay (Fig. 5, *B* and *D*). In the Mv1Lu cultures, cells bordering the scratch wound had prominent microfilament fibers running parallel to the wound (Fig. 5 *A*), while cells moving into the scratch wound redirected their microfilament assembly in the direction of cell movement (Fig. 5, *A* and *C*).

Cultures of contact-inhibited Mv1Lu cells maintained a tight cell monolayer for more than 8 d in serum-free medium indicative of cell cycle arrest, while the GlcNAc-TV transfectants showed progressive degeneration of the monolayer, with denuded areas and piling up of cells (Fig. 6, *A–C*). This phenotype was intermediate for R2 cells, and most pronounced in the M1 transfectant which had the highest levels of GlcNAc-TV activity. Mv1Lu cultures retained a greater number of viable cells, while M1 cultures showed accelerated rates of cell death after 3–8 d in serum-free medium. Cell death was characteristic of an apoptotic process as indicated by cell morphology and the characteristic fragmentation pattern of nuclear DNA (Fig. 6 *D*).

Substratum Cell Adhesion

Loss of intracellular adhesion (Bates et al., 1994) or cell-substratum adhesion has been shown to promote apoptosis in cultured nontransformed cells (Kim et al., 1994) and in mouse mammary gland during postlactating involution (Lefebvre et al., 1994). Therefore, GlcNAc-TV expression in Mv1Lu cells may alter cell–cell or cell–substratum adhesions which contribute to the observed phenotype of the transfectants; specifically, enhanced cell motility, reduced contact-inhibition, and sensitivity to apoptosis. As shown in Fig. 7, the transfectants were observed to be less adhesive to fibronectin- and collagen type IV–coated plastic. Similar results were obtained for Mv1Lu cells transiently transfected with a GlcNAc-TV expression vector (data not shown). Decreased cell–substratum adhesion did not appear to be due to loss of fibronectin or vitronectin receptors in the GlcNAc-TV transfectants (Fig. 7). $\alpha_5\beta_1$ and $\alpha_v\beta_3$ integrin levels on the cell surface were unchanged in

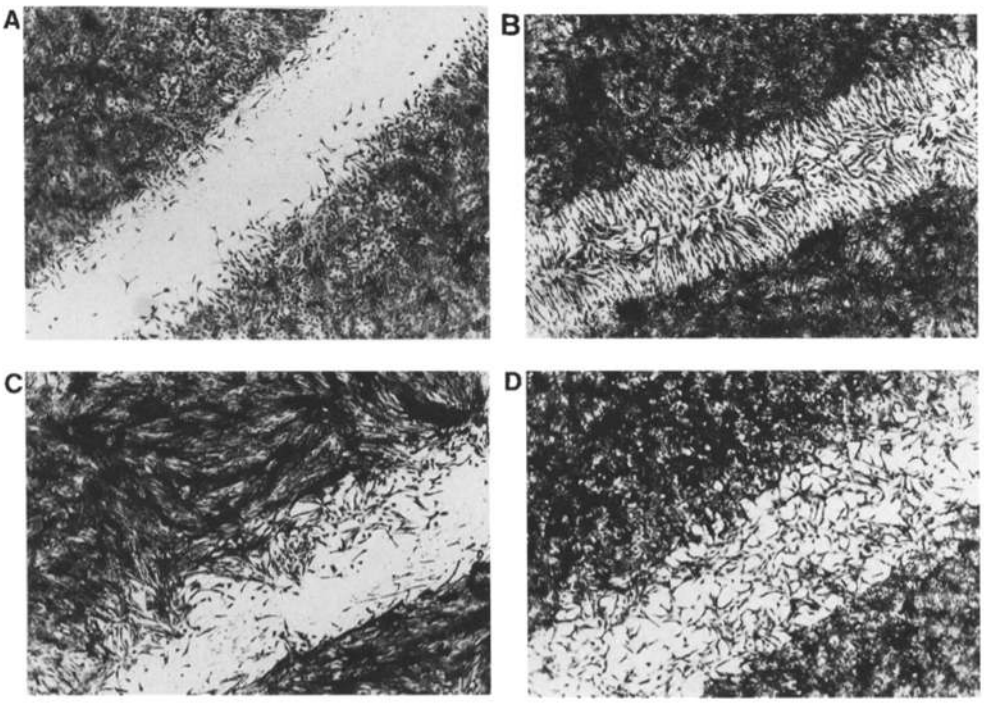

Figure 3. Repair of wounds in contact-inhibited monolayers of Mv1Lu cells and GlcNAc-TV transfectants. Confluent monolayers of cells were maintained in serum-containing α-MEM for at least 7 d, and then in serum-free α-MEM for 24 h followed by scraping a lane through the monolayer of cells with a plastic pipette tip. The cells were cultured in serum-free medium for an additional 24 h and then fixed, stained, and photographed. (*A*) C1; (*B*) M9; (*C*) M1; (*D*) R9.

the transfectants, however, the α_v, α_5, and β_1 integrin subunits from M1 and M9 cells migrated more slowly in the SDS-PAGE gels compared to that of Mv1Lu and R9, which correlated well with GlcNAc-TV enzyme levels in these cell lines. This data and work by others (Chammas et al., 1993; Zheng et al., 1994) suggest that a portion of the N-linked oligosaccharides on the α_5 and β_1 integrin glycoproteins serve as substrates for GlcNAc-TV in the transfectants.

Discussion

GlcNAc-TV activity (Yamashita et al., 1985; Lu and Chaney, 1993; Palcic et al., 1990) and mRNA levels (Miyoshi et al., 1993) increase after malignant transformation (Yamashita et al., 1985; Lu and Chaney, 1993; Palcic et al., 1990), with tumor progression (Dennis et al., 1987, 1989), and in premalignant hepatitis and hepatocarcinoma (Miyoshi et al., 1993). To study the effects of GlcNAc-TV expression on cellular phenotype, we have transfected Mv1Lu lung epithelial cells with a CMV-driven GlcNAc-TV expression vector and examined cell lines with 2.5–18 times more enzyme activity. GlcNAc-TV levels in the transfectants correlated with increased β1–6GlcNAc branching of oligosaccharides on LAMP-2 and cell surface integrin glycoproteins. At the cellular level, GlcNAc-TV expression promoted features of transformation including, releases from contact-inhibition of cell growth and reduced substratum adhesion, motility and increased tumorigenicity in nude mice. This phenotype bears similarity to primary mouse fibroblasts transfected with either *H-ras* (Tanaka et al., 1994) or c-*myc* (Evan et al., 1992). The cells are not fully transformed without an additional oncogenic event (e.g., loss of P53 or IRF-1), but are less contact-inhibited and die by apoptosis in low serum and high cell density conditions. In cultures of nontransformed keratinocytes and endothelial cells, substratum adhesion, and cell spreading is required for proliferation (Barrandon and Green, 1987; Sato and Rifkin, 1988; Ingber, 1990), and to prevent cell death by apoptosis (Re et al., 1994). Therefore, the reduced adhesiveness of the GlcNAc-TV transfected cells to fibronectin- and collagen-coated surfaces may contribute to foci formation in high density cultures, as well as apoptosis.

Table II. Contact-Inhibition and Cell Motility

Cell line	Cell migrated into the wound at 24 h	Percent of wound covered at 24 h
	cells/grid ± SD	
Mv1Lu	13 ± 4	5–10%
C1	16 ± 6	5–10%
R2	159 ± 11	>90%
R9	78 ± 7	70%
M9	103 ± 7	>90%
M1	56 ± 8	60%

Cells in the scratch wound were counted 24 h after wounding using a grid and photographs of the cultures. An estimate of the scratch wound area that had been covered by the migating cells was also made using the diameter of the wound before and after the 24-h repair period.

Repair or filling of the scratch wound involves both cell migration and proliferation, which appear to be interdependent processes with motility preceding entry into the cell cycle (Barrandon and Green, 1987). Unlike Mv1Lu and C1 cells, GlcNAc-TV transfected cells migrated rapidly into the scratch wounds, and had well developed microfilament and focal adhesions. Cell motility is a dynamic process requiring the turn over of focal adhesions and a balance between adhesive and de-adhesive forces. For example, antibodies to α_5 that marginally reduce substratum adhesion have been shown to enhance cell motility, while a complete blockade of integrins using anti-β_1 antibodies inhibited both adhesion and motility (Akiyama et al.,

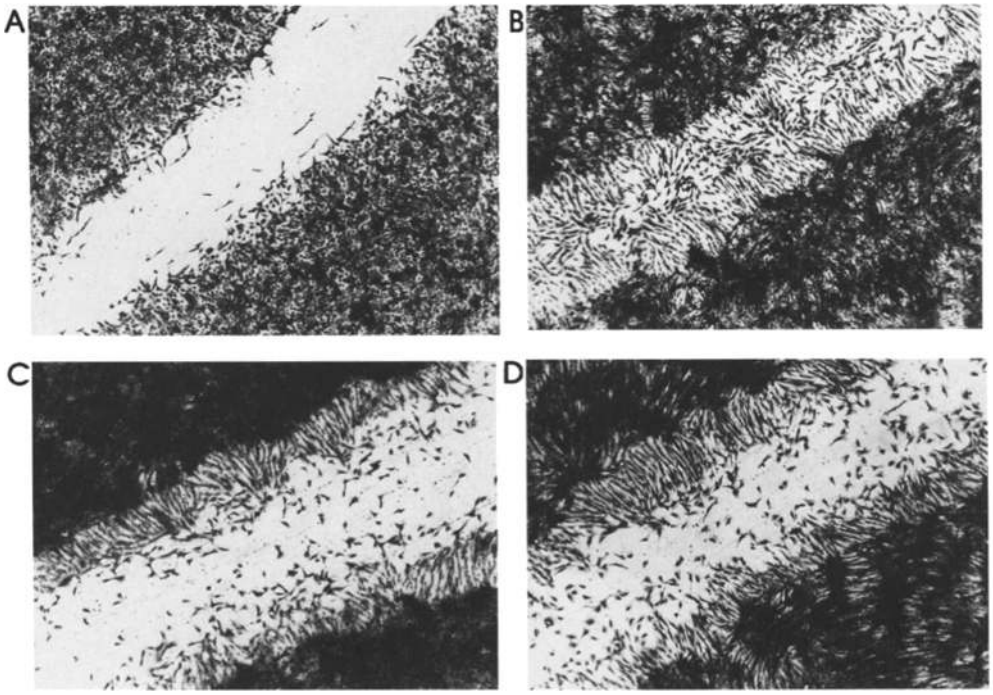

Figure 4. Swainsonine and TGFβ1 slow repair of wounds in contact-inhibited monolayers of GlcNAc-TV transfected Mv1Lu cells. (*A*) Mv1Lu; (*B*) M9; (*C*) M9 in 1 μg/ml of SW; (*D*) M9 in 50 pm of TGFβ1. Swainsonine also slowed migration of R9 and R2 transfectants into scratch wounds. Two independent experiments were done in duplicate and produced similar results.

1989*a*). Glycoconjugates including hyaluronic acid (Hardwick et al., 1992), proteoglycans (Bidanset et al., 1992), and laminin (Calof and Lander, 1991) have also been shown to reduce substratum adhesion and increase cell migration.

In the Mv1Lu transfected cells, LAMP glycoproteins and integrins were observed to be substrates of GlcNAc-TV, although the cell surface levels of the glycoproteins were unaffected. Altered glycosylation in the GlcNAc-TV transfected cells may affect cell adhesion through several

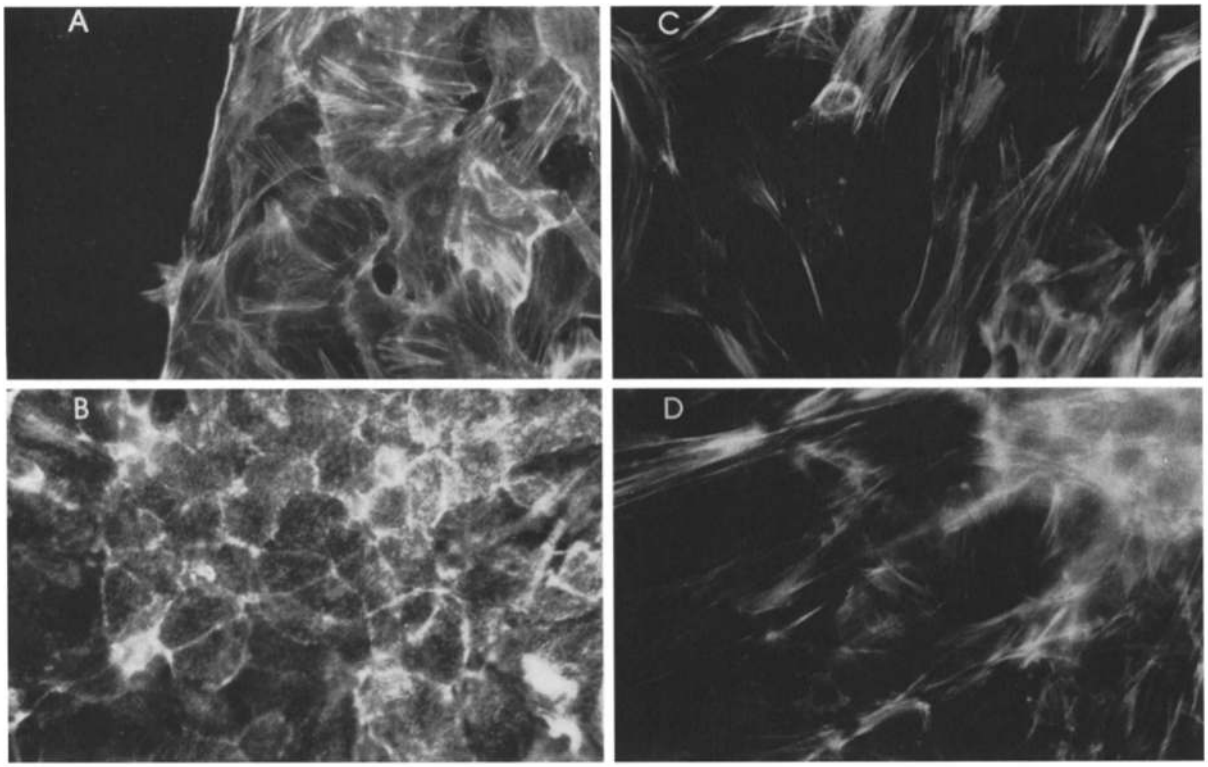

Figure 5. Microfilament structure in Mv1Lu cells and M1 cells. Confluent monolayers of cells were maintained in serum-containing α-MEM for 7 d and then in serum-free α-MEM for 24 h followed by scraping a lane. The cells were fixed 24 h later and stained with TRITC-labeled phalloidin as described in Materials and Methods. Mv1Lu cells (*A*) at boarder of scratch wound and (*B*) undisturbed monolayer; M1 cells (*C*) at boarder of scratch wound and (*D*) undisturbed monolayer. Note the foci in the upper right corner of *D*.

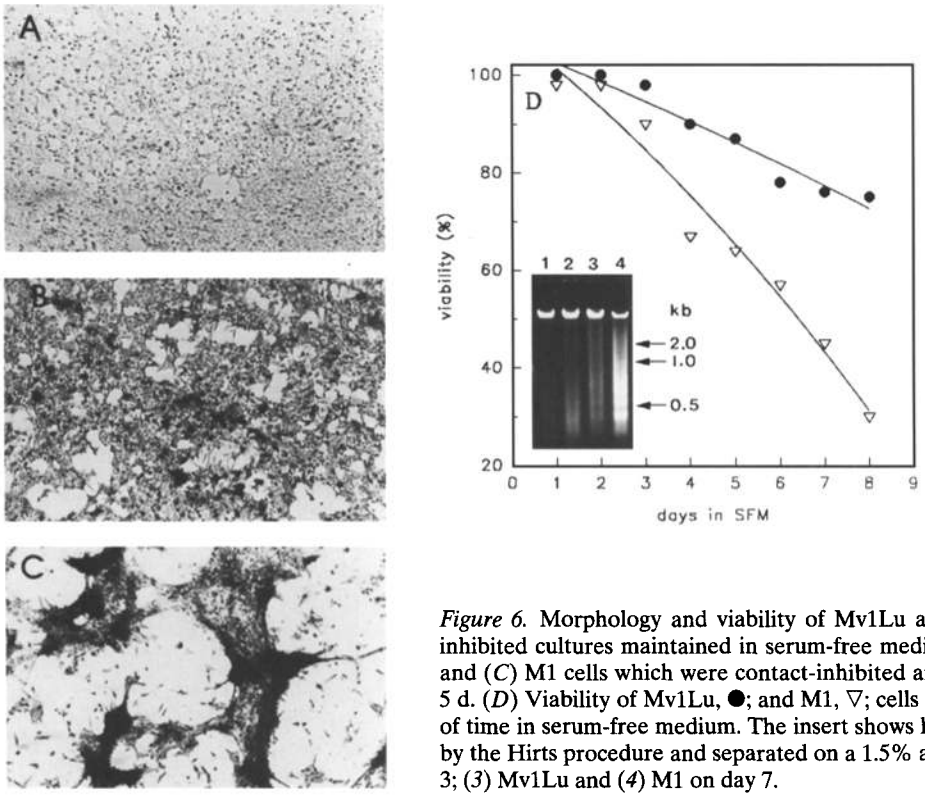

Figure 6. Morphology and viability of Mv1Lu and GlcNAc-TV transfectants in contact-inhibited cultures maintained in serum-free medium. Morphology of (*A*) Mv1Lu, (*B*) R2, and (*C*) M1 cells which were contact-inhibited and maintained in serum-free medium for 5 d. (*D*) Viability of Mv1Lu, ●; and M1, ▽; cells in contact-inhibited cultures as a function of time in serum-free medium. The insert shows low molecular weight DNA was extracted by the Hirts procedure and separated on a 1.5% agarose gel. (*1*) Mv1Lu and (*2*) M1 on day 3; (*3*) Mv1Lu and (*4*) M1 on day 7.

possible mechanisms, including integrin function or access to matrix, changes in matrix organization, and through lectin-carbohydrate interactions. The fibronectin-binding activity of $\alpha_5\beta_1$ has been shown to require maturation of N-linked oligosaccharides to complex-type chains (Akiyama et al., 1989*b*). Glycosylation often masks peptide domains in glycoproteins, which can enhance protease resistance, obscure antigenic domains and reduce receptor-ligand binding affinities (for reviews see Olden et al., 1985; Parekh, 1991; Varki, 1993). As such, the β1–6GlcNAc-branched oligosaccharides on either integrins or matrix proteins may reduce integrin-substratum binding or alter extracellular matrix organization and increase the availability of growth factors that are normally sequestered in the matrix. For example, the branched N-linked oligosaccharides and polylactosamine of embryonic fibronectin have been shown to reduce its affinity for collagen type I (Zhu et al., 1990, 1984). Similarly, the glycosylation of LAMP-1 in transformed cells has been shown to reduce its binding to collagen and fibronectin in vitro (Laferte and Dennis, 1988). LAMPs in tumor cells may serve to reduce cell–substratum adhesion, as a fraction of cellular LAMP is found on the basalateral surface of polarized epithelial cells in the course of it transits from the endoplasmic reticulum to the lysosomes (Nabi et al., 1991). GlcNAc-TV expression increases the lactosamine content in LAMP and other glycoproteins which can bind to the galactin family of lectins (Do et al., 1990; Cornil et al., 1990), and thereby may qualitatively change cell adhesion to favor carbohydrate-lectin interactions.

Cell adhesion, or more likely the turn over of focal adhesions can initiate intracellular signals which may act cooperatively with growth factor–induced signaling through the Ras signaling pathways. Specifically, integrin-mediated cell attachment induces autophosphorylation of focal adhesion kinase (Fak) creating a phosphopeptide domain that binds to the SH2-domain of $pp60^{src}$ (Schwartz et al., 1989; Ferrell and Martin, 1989; Guan and Shalloway, 1992; Schaller et al., 1994). $pp60^{src}$ also binds to Shc (Dilworth et al., 1994) and may in this manner couple cell adhesion–dependent signals to the Ras signaling pathway. In fact $p21^{ras}$ has been shown to be activated upon ligation of β1 integrins (Kapron-Bras et al., 1993). In a growth factor–depleted environment, structural features of the cell surface or matrix glycoproteins such as β1–6GlcNAc-branched oligosaccharides may facilitate the turn over of focal contacts and be a determining factor for intracellular signals that relieves contact-inhibition of cell growth. The present results, combined with earlier studies showing that transfection with activated-*ras* commonly induces GlcNAc-TV expression (Yamashita et al., 1985; Dennis et al., 1987, 1989; Lu and Chaney, 1993; Palcic et al., 1990), suggest the β1–6-branched oligosaccharides may be part of a postive feedback loop in the Ras-signaling cascade.

The processing of N-linked carbohydrates on certain growth factors (e.g., erythropoeitin, GM-CSF, IL4) and growth factor receptors (e.g., for insulin, interferonγ) affects their activity (for a review see Varki, 1993). Therefore, the phenotype of the GlcNAc-TV expressing cells might be due in part to enhanced autocrine growth stimulation. In this regard, GlcNAc-TV-deficient mutants and swainsonine-treated MDAY-D2 tumor cells show reduced responses to autocrine growth factors (Dennis et al., 1990; VanderElst and Dennis, 1991). Furthermore, growth factor and adhesion signals may be coupled, as indicated by a recent observation that insulin stimulation promotes asso-

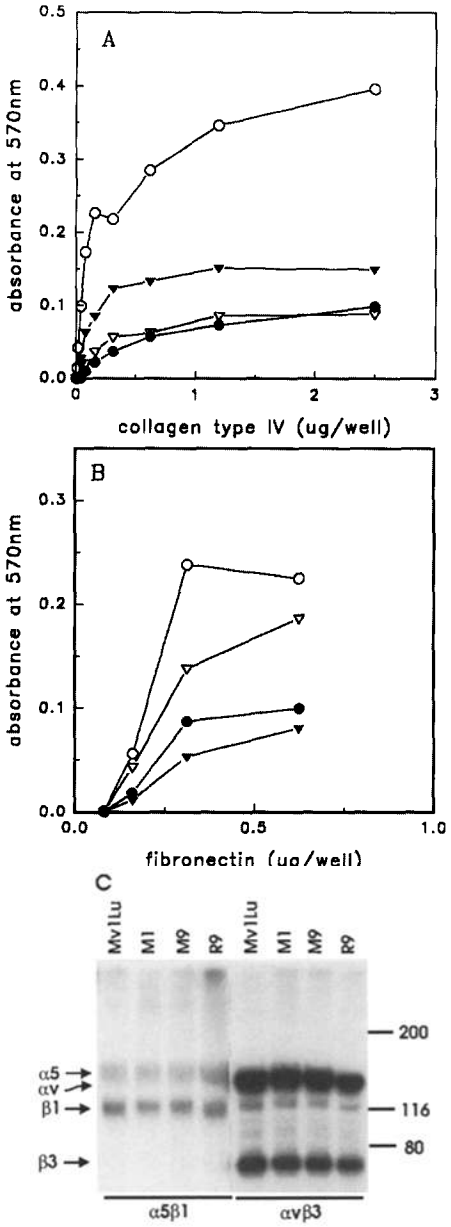

Figure 7. Cell attachment to fibronectin and collagen type IV. Mv1Lu cells and GlcNAc-TV transfectants from log phase cultures were applied to (*A*) collagen type IV–coated or (*B*) fibronectin-coated 96-well plates for 90 and 30 min, respectively, at 37°C. The results are duplicate measurements and representative of three experiments. The symbols are Mv1Lu, ○; M1, ▼; R9, ●; M9, ▽. (*C*) Immunoprecipitation of ^{125}I-labeled cell surface fibronectin receptor $\alpha_5\beta_1$ and the vitronectin $\alpha_v\beta_3$ from Mu1Lv and the transfectants.

ciation of $\alpha_v\beta_3$ with insulin receptor substrate (IRS-1), which can mediate signaling by binding to Grb-2 and PIK-3 kinase (Vuori and Ruoslahti, 1994).

Finally, GlcNAc-TV expression in normal mouse tissues is consistent with a role in cell migration and proliferation. β1–6GlcNAc branched N-linked oligosaccharides are found predominantly in cells capable of proliferation and motility. This includes trophoblasts, endothelial cells, interstitial fibroblasts, and activated haematopoietic cells (Fernandes et al., 1991; Lemaire et al., 1994). Furthermore, hepatocyte growth factor (HGF) which stimulates MDCK cell motility, also induces a fivefold increase in GlcNAc-TV activity in these cells (Warren, C., R. Nabi, M. Park, and J. Dennis, manuscript in preparation). In situ analysis of mouse embryos using antisense RNA probes revealed low GlcNAc-TV expression at embryonic day 7.5, and much greater levels at 9.5 d when rapid growth and organogenesis occurs. GlcNAc-TV expression is more restricted thereafter, and at embryonic day 17.5 is found in the crypt cells of intestine, epithelial cells of skin as well as neuroepithelium of the brain (Granowski, M., C. Fode, C. Warren, R. Campbell, J. Marth, M. Pierce, N. Fregien, and J. Dennis, manuscript in preparation). The basal cells of skin and intestine proliferate, and the progeny cells migrate or stratify, and finally undergo programmed cell death. Although cell proliferation is minimal in the intermediate zone of the neuroepithelium and the spinal column of the 17.5 day embryo, these are regions of active neuronal process migration, where apoptosis determines the correct size of sensory neural area and that of its targets.

In summary, expression of GlcNAc-TV in epithelial cells promotes features of transformation, including reduced substratum adhesion and releases from contact-inhibition of cell growth. These results show that in addition to promoting metastasis in the latter stages of tumor progression (Dennis et al., 1987), oncogene-induced upregulation of GlcNAc-TV in premalignant epithelial cells may contribute to loss of contact-inhibition of cell growth.

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References

Akiyama, S. K., S. S. Yamada, W.-T. Chen, and K. M. Yamada. 1989*a*. Analysis of fibronectin receptor function with monoclonal antibodies: roles in cell adhesion, migration, matrix assembly, and cytoskeletal organization. *J. Cell Biol.* 109:863–875.

Akiyama, S. K., S. S. Yamada, and K. M. Yamada. 1989*b*. Analysis of the role of glycosylation of the human fibronectin receptor. *J. Biol. Chem.* 264: 18011–18018.

Barrandon, Y., and H. Green. 1987. Cell migration is essential for sustained growth of keratinocyte colonies: the roles of transforming growth factor-β and epidermal growth factor. *Cell.* 50:1131–1137.

Bates, R. C., A. Buret, D. F. van Helden, M. A. Horton, and G. F. Burns. 1994. Apoptosis induced by inhibition of intercellular contact. *J. Cell Biol.* 125: 403–415.

Bidanset, D. J., R. LeBaron, L. Rosenberg, J. E. Murphy-Ullrich, and M. Hook. 1992. Regulation of cell substrate adhesion: effects of small galactosaminoglycan-containing proteoglycans. *J. Cell Biol.* 118:1523–1531.

Burridge, K., K. Fath, T. Kelly, G. Nuckolls, and C. Turner. 1988. Focal adhesions: transmembrane junctions between the extracellular matrix and cytoskeleton. *Annu. Rev. Cell. Biol.* 4:487–525.

Calof, A. L., and A. D. Lander. 1991. Relationship between neuronal migration and cell-substratum adhesion: laminin and merosine promote olfactory neuronal migration but are anti-adhesive. *J. Cell Biol.* 115:779–794.

Chammas, R., S. S. Veiga, L. R. Travassos, and R. R. Brentani. 1993. Functionally distinct roles for glycosylation of α and β integrin chains in cell-matrix interactions. *Proc. Natl. Acad. Sci. USA* 90:1795–1799.

Cornil, I., R. S. Kerbel, and J. W. Dennis. 1990. Tumor cell surface β1-4 linked galactose binds to lectin(s) on microvascular endothelial cells and contributes to organ colonization. *J. Cell Biol.* 111:773–782.

Cummings, R. D., and S. Kornfeld. 1982. Characterization of the structural determinants required for the high affinity interaction of asparagine-linked oligosaccharides with immobilized *Phaseolus vulgaris* leukoagglutinating and erthroagglutinating lectins. *J. Biol. Chem.* 257:11230–11234.

Dedhar, S., K. Robertson, and V. Gray. 1991. Induction of expression of αvβ1 and αvβ3 integrin heterodimers during retinoic acid-induced neural differentiation of murine embryonal carcinoma cells. *J. Biol. Chem.* 266:21846–21852.

Dennis, J. W. 1986. Different metastatic phenotypes in two genetic classes of WGA-resistant tumor cell mutants. *Cancer Res.* 46:4594–4600.

Dennis, J. W., S. Laferte, C. Waghorne, M. L. Breitman, and R. S. Kerbel. 1987. β 1-6 branching of Asn-linked oligosaccharides is directly associated with metastasis. *Science (Wash. DC).* 236:582–585.

Dennis, J. W., K. Kosh, D.-M. Bryce, and M. Breitman. 1989. Oncogenes conferring metastatic potential induce increased branching of Asn-linked oligosaccharides in rat2 fibroblasts. *Oncogene.* 4:853–860.

Dennis, J. W., K. Koch, S. Yousefi, and I. VanderElst. 1990. Growth inhibition of human melanoma tumor xenografts in athymic nude mice by swainsonine. *Cancer Res.* 50:1867–1872.

DeSantis, R., U. V. Santer, and M. C. Glick. 1987. NIH 3T3 cells transfected with human tumor DNA lose the transformed phenotype when treated with swainsonine. *Biochem. Biophys. Res. Commun.* 142:348–353.

Dilworth, S. M., C. E. P. Brewster, M. D. Jones, L. Lanfrancone, G. Pelicci, and P. G. Pelicci. 1994. Transformation by polyoma virus middle T-antigen involves the binding and tyrosine phosphorylation of Shc. *Nature (Lond.).* 367: 87–90.

Do, K.-Y., D. F. Smith, and R. D. Cummings. 1990. Lamp-1 in CHO cells is a primary carrier of poly-*n*-acetyllactosamine chains and is bound preferentially by a mammalian S-type lectin. *Biochem. Biophys. Res. Commun.* 173: 1123–1128.

Dulbecco, R. 1970. Topoinhibition and serum requirement of transformed and untransformed cells. *Nature (Lond.).* 227:802–806.

Evan, G. I., A. H. Wyllie, C. S. Gilbert, T. D. Littlewood, H. Land, M. Brooks, C. M. Waters, L. Z. Penn, and D. C. Hancock. 1992. Induction of apoptosis in fibroblasts by c-myc protein. *Cell.* 69:119–128.

Ewen, M. E., H. K. Sluss, L. L. Whitehouse, and D. M. Livingston. 1993. TGF-β inhibition of cdk4 synthesis is linked to cells cycle arrest. *Cell.* 74:1009–1020.

Fernandes, B., U. Sagman, M. Auger, M. Demetrio, and J. W. Dennis. 1991. β1–6 branched oligosaccharides as a marker of tumor progression in human breast and colon neoplasia. *Cancer Res.* 51:718–723.

Ferrell, J. E., Jr., and G. S. Martin. 1989. Tyrosine-specific protein phosphorylation is regulated by glycoprotein IIb-IIIa in platelets. *Proc. Natl. Acad. Sci. USA* 86:2234–2238.

Fukuda, M., J. Vitala, J. Matteson, and S. R. Carlson. 1988. Cloning of cDNAs encoding human lysosomal membrane glycoproteins, LAMP-1 and LAMP-2. *J. Biol. Chem.* 236:18920–18928.

Guan, J.-L., and D. Shalloway. 1992. Regulation of focal adhesion-associated protein tyrosine kinase by both cellular adhesion and oncogenic transformation. *Nature (Lond.).* 358:690–692.

Hadwiger, A., H. Niemann, A. Kabisch, H. Bauer, and T. Tamura. 1986. Appropriate glycosylation of the fms gene product is a prerequisite for its transforming potency. *EMBO (Eur. Mol. Biol. Organ.) J.* 5:689–694.

Hakomori, S.-I. 1989. Aberrant glycosylation in tumors and tumor-associated carbohydrate antigens. *Adv. Cancer Res.* 52:257–331.

Hardwick, C., K. Hoare, R. Owens, H. P. Hohn, M. Hook, D. Moore, V. Cripps, L. Austen, D. M. Nance, and E. A. Turley. 1992. Molecular cloning of a novel hyaluronan receptor that mediates tumor cell motility. *J. Cell Biol.* 117:1343–1350.

Heffernan, M., and J. W. Dennis. 1989. Molecular characterization of P2B/LAMP-1, a major protein target of a metastasis-associated oligosaccharide structure. *Cancer Res.* 49:6077–6084.

Heffernan, M., R. Lotan, B. Amos, M. Palcic, Y. Takano, and J. W. Dennis. 1993. Branching β1–6*N*-Acetylglucosaminetransferases and polylactosamine expression in mouse F9 teratocarcinoma cells and differentiated counterparts. *J. Biol. Chem.* 268:1242–1251.

Hirt, B. 1967. Selective extraction of polyoma DNA from infected mouse cell cultures. *J. Mol. Biol.* 26:365–369.

Ingber, D. E. 1990. Fibronectin controls capillary endothelial cell growth by modulating cell shape. *Proc. Natl. Acad. Sci. USA.* 87:3579–3583.

Itzkowitz, S. H., M. Yuan, Y. Fukushi, A. Palekar, P. C. Phelphs, A. M. Shamsuddin, B. F. Trump, S. Hakomori, and Y. S. Kim. 1986. $Lewis^X$ and sialylated $Lewis^X$-related antigen expression in human malignant and non-malignant colonic tissue. *Cancer Res.* 46:2627–2632.

Kapron-Bras, C., L. Fitz-Gibbon, P. Jeevaratnam, J. Wilkins, and S. Dedhar. 1993. Stimulation of tyrosine phosphorylation and accumulation of GTP-bound p21ras upon antibody-mediated α2β1 integrin activation in T-lympholastic cells. *J. Biol. Chem.* 268:20701–20704.

Khan, M. Z., D. A. Spandidos, D. J. Kerr, A. M. McNicol, J. C. Lang, L. De Ridder, and R. I. Freshney. 1991. Oncogene transfection of mink lung cells: effect on growth characteristics *in vitro* and *in vivo*. *Anticancer Res.* 11:1343–1348.

Kim, W. H., W. Schnaper, M. Nomizu, Y. Yamada, and H. K. Kleinman. 1994. Apoptosis in human fibrosarcoma cells is induced by a multimeric synthetic Tyr-Ile-Gly-Ser-Arg (YIGSR)-containing polypeptide from laminin. *Cancer Res.* 54:5005–5010.

Kim, Y. S., M. Yuan, S. H. Itzkowitz, Q. Sun, T. Kaizu, A. Palekar, B. F. Trump, and S. Hakomori. 1986. Expression of Le^Y and extended Le^Y blood group-related antigens in human malignant, premalignant, and nonmalignant colonic tissues. *Cancer Res.* 46:5985–5992.

Knight, J., H. Beug, J. Marshall, and M. J. Hayman. 1988. Abnormal glycosylation of the *env-sea* oncogene product inhibits its proteolytic cleavage and blocks its transforming ability. *Oncogene.* 2:317–326.

Kornfeld, R., and S. Kornfeld. 1985. Assembly of asparagine-linked oligosaccharides. *Annu. Rev. Biochem.* 54:631–664.

Laferte, S., and J. W. Dennis. 1988. Glycosylation-dependent collagen-binding activities of two membrane glycoproteins. *Cancer Res.* 48:4743–4748.

Laferte, S., and J. W. Dennis. 1989. Purification of two glycoproteins expressing β1–6 branched Asn-linked oligosaccharides commonly associated with the malignant phenotype. *Biochem. J.* 259:569–576.

Lefebvre, O., C. Wolf, J.-M. Limacher, P. Hutin, C. Wendling, M. LeMeur, P. Basset, and M.-C. Rio. 1994. The breast cancer-associated stromelysin-3 gene is expressed during mouse mammary gland apoptosis. *J. Cell Biol.* 119: 997–1002.

Lemaire, S., C. Derappe, J. C. Michalski, M. Aubery, and D. Neel. 1994. Expression of β1–6-branchied N-linked oligosaccharides is associated with activation in human T4 and T8 cell populations. *J. Biol. Chem.* 269:8069–8074.

Lu, Y., and W. Chaney. 1993. Induction of N-acetylglucosaminyltransferase V by elevated expression of activated or proto-Ha-ras oncogenes. *Mol. Cell. Biochem.* 122:85–92.

Miyoshi, E., A. Nishikawa, Y. Ihara, J. Gu, T. Sugiyama, N. Hayashi, H. Fusamoto, T. Kamada, and N. Taniguchi. 1993. *N*-acetylglucosaminyltransferase III and V messenger RNA levels in LEC rats during hepatocarcinogenesis. *Cancer Res.* 53:3899–3902.

Nabi, I. R., A. Le Bivic, D. Fambrough, and E. Rodriguez-Boulan. 1991. An endogenous MDCK lysosomal membrane glycoprotein is targeted basolaterally before delivery to lysosomes. *J. Cell Biol.* 115:1573–1584.

Nabi, I. R., and E. Rodriguez-Boulan. 1993. Increased LAMP-2 polylactosamine glycosylation is associated with its slower Golgi transit during establishment of a polarized MDCK epithelial monolayer. *Mol. Biol. Cell.* 4: 627–635.

Nichols, E. J., R. Manger, S. Hakomori, A. Herscovics, and L. R. Rohrschneider. 1985. Transformation by the v-fms oncogene product: role of glycosylational processing and cell surface expression. *Mol. Cell. Biol.* 5:3467–3475.

Olden, K., B. A. Bernard, M. J. Humphries, T.-K. Yeo, K.-T. Yeo, S. L. White, S. A. Newton, H. C. Bauer, and J. B. Parent. 1985. Function of glycoprotein glycans. *TIBS.* 10:78–82.

Palcic, M. M., J. Ripka, K. J. Kaur, M. Shoreibah, O. Hindsgaul, and M. Pierce. 1990. Regulation of N-acetylglucosaminyltransferase V activity. *J. Biol. Chem.* 265:6759–6769.

Parekh, R. B. 1991. Effects of glycosylation on protein function. *Curr. Opin. Struct. Biol.* 1:750–754.

Paulson, J. C., J. Weinstein, and A. Schauer. 1989. Tissue-specific expression of sialyltransferases. *J. Biol. Chem.* 264:10931–10934.

Re, F., A. Zanetti, M. Sironi, N. Polentarutti, L. Lanfracone, E. Dejana, and F. Colotta. 1994. Inhibition of anchorage-dependent cell spreading triggers apoptosis in cultured human endothelial cells. *J. Cell Biol.* 127:537–546.

Sato, Y., and D. B. Rifkin. 1988. Autocrine activities of basic fibroblast growth factor: regulation of endothelial cell movement, plasminogen activator synthesis, and DNA synthesis. *J. Cell Biol.* 107:1199–1205.

Schachter, H. 1986. Biosynthetic controls that determine the branching and microheterogeneity of protein-bound oligosaccharides. *Biochem. Cell Biol.* 64: 163–181.

Schaller, M. D., J. D. Hildebrand, J. D. Shannon, J. W. Fox, R. R. Vines, and J. T. Parsons. 1994. Autophosphorylation of the focal adhesion kinase, $pp125^{FAK}$, directs SH2-dependent binding of $pp60^{src}$. *Mol. Cell. Biol.* 14: 1680–1688.

Schwartz, M. A., G. Both, and C. Lechene. 1989. Effect of cell spreading on cytoplasmic pH in normal and transformed fibroblasts. *Proc. Natl. Acad. Sci. USA.* 86:4525–4529.

Seftor, R. E. B., E. A. Seftor, W. J. Grimes, L. A. Liotta, W. G. Stetler-Stevenson, D. R. Welch, and M. J. C. Hendrix. 1991. Human melanoma cell invasion is inhibited *in vitro* by swainsonine and deoxymannojirimycin with a concomitant decrease in collagenase IV expression. *Melanoma Res.* 1:43–54.

Shoreibah, M., G–S. Perng, B. Adler, J. Weinstein, R. Basu, R. Cupples, D. Wen, J. K. Browne, P. Buckhaults, N. Fregien, et al. 1993. Isolation, characterization, and expression of cDNA encoding *N*-Acetylglucosaminyltransferase V. *J. Biol. Chem.* 268:15381–15385.

Tanaka, N., M. Ishihara, M. Kitagawa, H. Harada, T. Kimura, T. Matsuyama, M. S. Lamphier, S. Aizawa, and T. W. Mak. 1994. Cellular commitment to oncogene-induced transformation or apoptosis is dependent on the transcription factor IRF-1. *Cell.* 77:829–839.

van den Eijnden, D. H., A. H. L. Koenderman, and W. E. C. M. Schiphorst. 1988. Biosynthesis of blood group i-active polylactosaminoglycans. *J. Biol. Chem.* 263:12461–12465.

VanderElst, I., and J. W. Dennis. 1991. N-linked oligosaccharide processing and autocrine-stimulation of tumor cell proliferation. *Exp. Cell Res.* 192:612–613.

Varki, A. 1993. Biological roles of oligosaccharides: all of the theories are correct. *Glycobiology.* 3:97–130.

Vuori, K., and E. Ruoslahti. 1994. Association of insulin receptor substrate-1

with integrins. *Science (Wash. DC)*. 266:1576–1578.

Yagel, S., R. Feinmesser, C. Waghorne, P. K. Lala, M. L. Breitman, and J. W. Dennis. 1989. Evidence that β 1-6 branched Asn-linked oligosaccharides on metastatic tumor cells facilitate invasion of basement membranes. *Int. J. Cancer*. 44:685–690.

Yamashita, K., Y. Tachibana, T. Ohkura, and A. Kobata. 1985. Enzymatic basis for the structural changes of asparagine-linked sugar chains of membrane glycoproteins of baby hamster kidney cells induced by polyoma transformation. *J. Biol. Chem.* 260:3963–3969.

Yousefi, S., E. Higgins, Z. Doaling, O. Hindsgaul, A. Pollex-Kruger, and J. W. Dennis. 1991. Increased UDP-GlcNAc:Gal β1-3GalNAc-R (GlcNAc to GalNAc) β1–6-N-acetylglucosaminyltransferase activity in transformed and metastatic murine tumor cell lines: control of polylactosamine-synthesis. *J. Biol. Chem.* 266:1772–1783.

Zheng, M., H. Fang, and S.-I. Hakomori. 1994. Functional role of *N*-glycosylation in α5β1 integrin receptor. *J. Biol. Chem.* 269:12325–12331.

Zhu, B. C.-R., S. F. Fisher, H. Pande, J. Calaycay, J. E. Shively, and R. A. Laine. 1984. Human placental (fetal) fibronectin: increased glycosylation and higher protease resistance than plasma fibronectin. *J. Biol. Chem.* 259: 3962–3970.

Zhu, B. C. R., R. A. Laine, and M. D. Barkley. 1990. Intrinsic tryptophan fluorescence measurements suggest that polylactosaminyl glycosylation affects the protein conformation of the gelatin-binding domain from human placental fibronectin. *Eur. J. Biochem.* 189:509–516.