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Differential expression of an α-galactosyl-containing trisaccharide on high- and low-malignant murine sarcoma cells: Identification and regulation

James Varani[1], Jerzy Petryniak[2], Masaru Takagaki[2], Michael K. Dame[1], Bronislawa Petryniak[2] & Irwin J. Goldstein[2]

Departments of [1]Pathology and [2]Biological Chemistry, The University of Michigan Medical School, Ann Arbor, Michigan, USA

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Abstract

Past studies have shown that carbohydrate residues reactive with the *Griffonia simplicifolia* isolectin B_4 (GS I-B4) are present on the surface of highly-malignant murine sarcoma cells but are lacking or expressed in much lower amounts on the surface of low-malignant cells isolated from the same parent tumors (Am J Pathol 111: 27; J Nat Cancer Inst 71: 1281). In the present study it is shown that an antibody which recognizes the trisaccharide Galα1-3Galβ1-4GlcNAc- is reactive with the highly-malignant cells but is non-reactive with the low-malignant cells. Further studies show that the high-malignant cells not only bind GS I-B_4 but also bind *Evonymus europaea* lectin (which like GS I-B_4 recognizes terminal galactose in α1-3 linkage) and *Erythina crystagalli* lectin (which recognizes sub-terminal galactose in the β1-4 linkage – e.g., Galβ1-4GlcNAc). In contrast, the low malignant cells bind *Erythina crystagalli* lectin as efficiently as the high malignant cells but do not bind (or bind much smaller amounts of) either GS I-B_4 or *Evonymus europaea* lectin. The present studies also show that there is no significant difference between high- and low-malignant cells in expression of α-galactosidase activity. In contrast, the high-malignant cells express high levels of α-galactosyl transferase activity while this enzyme is virtually undetectable in low-malignant cells. Taken together, these studies indicate that differential expression of a single monosaccharide residue distinguishes high- and low-malignant murine sarcoma cells. These studies also identify a mechanism to account for surface carbohydrate differences between the high- and low-malignant cells.

Introduction

In previous studies we established a series of high-malignant and low-malignant cell lines from a number of different carcinogen-induced murine sarcomas [1, 2]. Among these tumor cell lines there was a 100% correlation between capacity of the cell lines to bind *Griffonia simplifolia* isolectin B_4 (GS I-B_4) and capacity to form metastatic tumors in syngeneic mice [2, 3]. GS I-B_4 recognizes carbohydrate structures that terminate in α-D-galacosyl residues (αGal) linked to its sub-terminal sugar [4]. Although GS I-B_4 is not completely specific for αGal, this monosaccharide is one of the preferred ligands [4]. Following our initial observations with the murine sarcoma cells, Grimstad and Bosnes extended these findings to other murine tumor lines [5]. Additionally, Castronovo et al. showed that a naturally occurring antibody which recognizes αGal not only reacted with murine MO4 cells, but also inhibited lung colonization by intravenously-injected MO4 cells [6].

Correspondence to: James Varani, PhD, Department of Pathology, The University of Michigan Medical School, 1301 Catherine Road/Box 0602, Ann Arbor, MI 48109, USA. Tel: +1-734-615-0298; Fax: +1-734-763-6476; E-mail: varani@umich.edu

Terminal αGal expression is rare in primates [7]. It is of interest, therefore, that this epitope has been detected on the surface of a number of human tumor lines including those derived from breast epithelium [8–10]. Consistent with this, it was shown by Petryniak et al. that human breast cancer cells but not normal breast epithelial cells are reactive with GS I-B_4 [11]. Since expression of epitopes that are reactive with GS I-B_4 is correlated with malignant potential in both rodents and humans, efforts to delineate the structural basis for lectin reactivity and efforts to understand how expression of these epitopes is regulated seem warranted. These issues are addressed in the present report.

Materials and methods

Cells

High- and low-malignant murine tumor cell lines were established from three different methylchonanthrene-induced sarcomas in C57bl/6 mice [1, 2]. The high-malignant cells were designated as 1.0, 1.1 and 1.2, respectively, while their counterpart low-malignant cells were designated as 1.0/anti-B^r, 1.1/anti-B^r and 1.2/anti-B^r respectively. Anti-B^r connotes resistance to lysis in the presence of complement

and antibodies to the human blood group B antigen [3]. For present studies, all of the cell lines were grown in monolayer culture using Minimal Essential Medium of Eagle with Earl's salts supplemented with non-essential amino acids (MEM) and 10% fetal bovine serum (MEM-FBS) as culture medium. Cells were grown at 37 °C and 5% CO_2 and sub-cultured as required.

Monoclonal antibody

We previously reported on the preparation of a monoclonal antibody which recognizes the epitope: Galα1-3Galβ1-4GlcNAc- [12]. In brief, plasma membrane glycoproteins from Ehrlich ascites tumor cells were isolated and used to immunize BALB/c mice. Cell fusion was carried out according to the methods of Galfre et al. and McKearn [13, 14]. Clones were screened by enzyme-linked immunosorbant assay with Galα1-3Galβ1-4GlcNAc-bovine serum albumin and Galα1-3Galβ1-4GlcNAc-bovine thyroglobulin as substrates. Only clones reactive with both substrates were expanded and subcloned. Stable cultures of antibody-producing cells were eventually obtained and monoclonal antibody was produced from these cells in pristane-treated BALB/c mice. The monoclonal antibody was purified by chromatography on a Galα1-3Galβ1-4GlcNAc-Synsorb affinity column.

Monoclonal antibody staining

Each of the cell lines was stained with the monoclonal antibody. To 50 μl of a suspension of washed cells in PBS containing 5% fetal bovine serum (PBS-FBS) was added 50 μl of the monoclonal antibody solution at 5 μg/ml in PBS. Normal mouse IgM was used at the same concentration as a control. After incubation for 30 min, the cells were washed and stained with 50 μl of FITC-coupled anti-mouse Ig(G+M) antibody (Boehringer Mannheim, Indianapolis, Indiana). The cells were then washed exhaustively in PBS-FBS and finally resuspended in the same buffer. They were examined by fluorescence microscopy and photographed.

Lectin staining

Lectin-staining was carried out with each of the cell lines. Lectins used included GS I-B_4, *Evonymus europaea* lectin and *Erythina cyrystagalli* lectin. The three lectins were obtained commercially (Sigma Chemical Co., St. Louis, Missouri and E-Y Laboratories, San Mateo, California) as fluorescein isothiocyanate (FITC) conjugates. Specific carbohydrate inhibitors for each of the sugars were also obtained from commercial sources. These include methyl α-D-galactopyranoside (10 mM) for GS I-B_4, N-acetyllactosamine (10 mM) for *Erythina crystagalli* and lactose (100 mM) for *Evonymus europaea*.

Cells in suspension after removal from the substratum by brief trypsinization were stained with each of the lectins. Briefly, 250,000 cells were mixed with 10 μg of the FITC-labeled lectins in a total volume of 150 μl of PBS. When inhibitors were present, they were added at a final concentration of 10–100 mM. After incubation for 45 min at room temperature, cells were separated from the unbound lectin, washed two times in PBS and fixed in 2% paraformaldeyde. The stained cells were then analyzed by flow cytometry. Routinely, 10,000 cells were analyzed per group.

α-Galactosidase treatment

In certain experiments, cells were exposed to green coffee bean α-galactosidase (Worthington Chemicals Corporation; Freehold, New Jersey) prior to staining. For this, 1.2 units of enzyme was incubated for three hours with 2,000,000 cells in a final volume of 0.5 ml. MEM-FBS was used as incubation buffer. After enzyme treatment, the cells were washed and stained as described above.

Galactosyl transferase measurement

Cell extracts were prepared from each of the high and low malignant cells and standardized with regard to protein concentration. Extracts were assayed for galactosyl transferase activity using transfer of UDP-^{14}C-galactose to asialo-orosomucoid as the galactose acceptor. Asialo-orosomucoid was used as the acceptor because olgiosaccharides of this glycoprotein terminate in Galα1-4GlcNAc. This disaccharide can accept D-galactose in the α position only. The reaction mixture (100 μl final volume) contained 20 mM HEPES, pH 6.2, 10 mM $MnCl_2$, 38 μg of cross-linked asialo-orosomucoid, 0.1 μCi UDP-^{14}C-galactose, 0.2% β-mercaptoethanol and varying amounts of cell lysates. [Cross-linked asialo-orosomucoid was prepared by mixing 5 ml of 0.1 M 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide (EDC) (Pierce Chemical Company, Rockford, Illinois) containing 5 mM sulfo-N-hydroxysuccinamide (sulfo-NHS) with 26.7 mg of asialo-orosomucoid at pH 8.5. After incubation for 15 h at 37 °C, the mixture was dialyzed exhaustively against water and then lyophilized until use.] The mixture for galactosyl transferase measurement was incubated for one hour at 37 °C. The reaction was terminated by adding 100 μl of 4% phosphotungstic acid in 20% trichloroacetic acid and incubation for 15 min in the cold. The precipitate which formed was centrifuged in a microfuge at maximal speed for 5 min, washed two times with 2% phosphotungstic acid in 10% trichloroacetic acid and solubilized in 350 μl of 1N NaOH for 2 h at 37 °C. The solution was then counted for total radioactivity by liquid scintillation. Controls included mixtures without cell extracts and mixtures without cross-linked asialo-orosomucoid. Values obtained in control samples without cell extracts were routinely less than 50 counts per minute while values obtained in the presence of cell extracts but without the asialo-orosomucoid were up to 1500 counts per minute.

Northern blot analysis for α1-3 galactosyl transferase mRNA

Total cellular RNA was extracted from one set of high and low malignant cells (1×10^7 1.0 and 1.0/anti-B^r cells) according to the procedure of Chirgwin et al. [41]. After

fractionation on a denaturing 1% formaldehyde-agarose gel, RNA was hybridized with ^{32}P-labeled pCDM7-murine α1-3 galactosyltransferase cDNA at high stringency. The murine α1-3 galactosyltransferase cDNA probe was a generous gift from Dr John Lowe (Department of Pathology, University of Michigan) [15].

Galactosidase measurement

Extracts prepared from each of the high and low malignant cells were assayed for α-galactosidase activity. The p-nitrophenyl derivative of α-D-galactopyranoside was used as substrate and the assay was conducted as described previously [1, 16]. Briefly, this involved incubation of the substrate with the extract for 1 h at 37 °C and pH 4.3. At the end of the incubation period, the sample was brought to pH 10.4 with 0.4 N glycine-NaOH buffer and the yellow color which developed at alkaline pH quantified spectrophotometrically at 405 nm. Preliminary experiments indicated that measurements made at one hour of incubation were on the linear portion of the enzyme activity curve.

Results

Reactivity of the high- and low-malignant murine sarcoma cells with an anti-trisaccharide monoclonal antibody

In the first series of experiment, the three high-malignant cell lines and three low-malignant cell lines were examined for reactivity with a monoclonal anti-trisaccharide antibody. Immunofluorescence photographs of one pair of high- and low-malignant cell lines (1.0 and 1.0/anti-B^r cells) are shown in Figure 1. The high malignant cells (1.0) shown in the left-panels were reactive with the anti-trisaccharide antibody (C) while the low malignant cells (1.0/anti B^r) (right panels) demonstrated much less staining (D). The other two pairs of tumor lines were identical to the 1.0 and 1.0/anti-B^r cells. None of the cells showed significant reactivity with the control IgM antibody (Figures 1A and B).

Lectin-reactivity of high and low malignant cells

The same three pairs of high-malignant and low-malignant cell lines were next examined for reactivity with a panel of lectins. Histograms of one pair of high- and low-malignant cell lines (1.0 and 1.0/anti-B^r cells) are shown in Figure 2. The high-malignant (1.0) cells were reactive with all three lectins while the low malignant (1.0/anti-B^r) cells demonstrated much less reactivity with GS I-B4 and Evonymus europaea lectin than did the high-malignant cells. In contrast, high- and low-malignant cells were equally reactive with *Erythina crystagalli* lectin. Data from all three pairs of cell lines were consistent with the findings presented in Figure 2, and these data are summarized in Table 1.

Effects of α-galactosidase treatment on lectin reactivity

High malignant cells (1.0) were exposed to green coffee bean α-galactosidase as described in the 'Materials and methods' section. Exposure of the cells to the enzyme for one hour under conditions which were non-toxic to the cells resulted in the loss of virtually 100% of the staining with GS I-B$_4$ (Figure 3). Staining with *Evonymus europaea* was also reduced following α-galactosidase treatment, but this treatment had no effect on *Erythina crystagalli* staining (not shown). Taken together with lectin- and antibody-staining data, these findings suggest that the high malignant cells express the trisaccharide Galα1-3Galβ1-4GlcNAc- on their surface. Further, these studies suggest that only the terminal monosaccharide unit (αGal) is missing on the low malignant cells, while there appears to be little difference in expression of subterminal Gal β1-4GlcNAc- between the high- and low-malignant cells.

Table 1. Lectin-reactivity of high and low malignant murine fibrosarcoma cells.

Cell line	Mean channel number		
	GS I-B$_4$	*Evonymus europaea*	*Erythina crystagalli*
1.0			
No lectin	5	4	3
Lectin	3919	327	326
Lectin + inhibitor	266	13	103
1.0/anti-B^r			
No lectin	6	7	6
Lectin	120	17	861
Lectin + inhibitor	69	7	208
1.1			
No lectin	4	5	4
Lectin	707	160	1194
Lectin + inhibitor	452	43	–
1.1/anti-B^r			
No lectin	4	5	6
Lectin	24	11	850
Lectin + inhibitor	10	8	190
1.2			
No lectin	5	5	5
Lectin	1227	263	324
Lectin + inhibitor	84	44	97
1.2/anti-B^r			
No lectin	4	5	4
Lectin	17	10	642
Lectin + inhibitor	10	8	450

Cells were treated with FITC-labeled lectins as described in the 'Materials and methods' section and examined by flow cytometry. Values shown represent mean channel fluorescence based on 10,000 independent measurements. 'No lectin' control represents background autofluorescence. 'Lectin' alone represents total fluorescence and 'Lectin + inhibitor' represents non-specific fluorescence. Inhibitor for GS I-B$_4$ was 10 mM methyl α-D-galactopyranoside; Inhibitor for *Evonymus europaea* was 100 mM lactose and inhibitor for *Erythina crystagalli* was 10 mM N-acetyllactosamine. Each cell line was examined on three or more occasions with similar results.

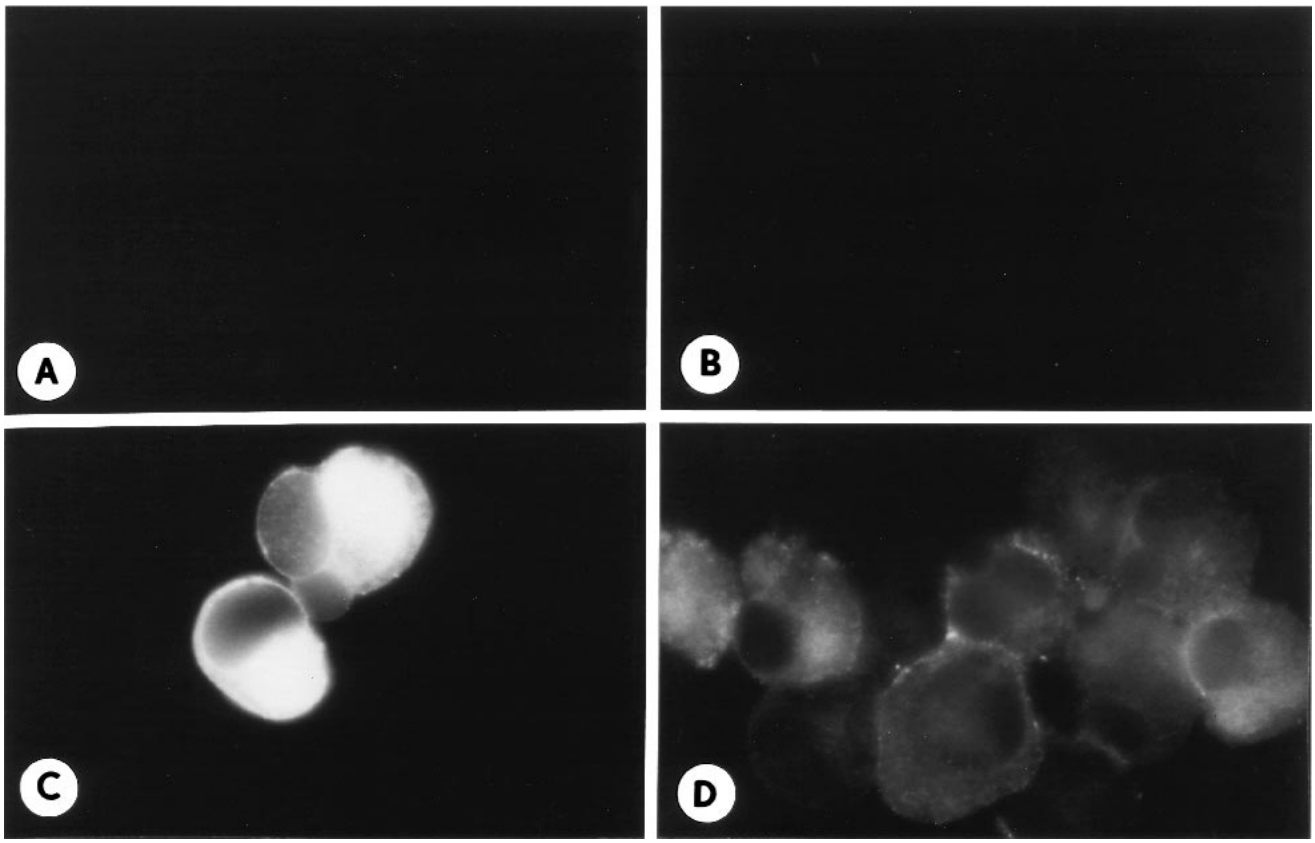

Figure 1. Detection of Gal α1-3 Gal β1-4 GlcNAc- on high-malignant and low-malignant murine fibrosarcoma cells by indirect immunofluorescence. Tumor cells were stained in suspension as described in the 'Materials and methods' section. Immunofluorescence photomicrographs of (A) high-malignant (1.0) cells and (B) low-malignant (1.0/anti-B^r) cells stained with control IgM. Immunofluorescence photomicrographs of (C) high-malignant (1.0) cells and (D) low-malignant (1.0/anti-B^r) cells stained with anti-trisaccharide antibody.

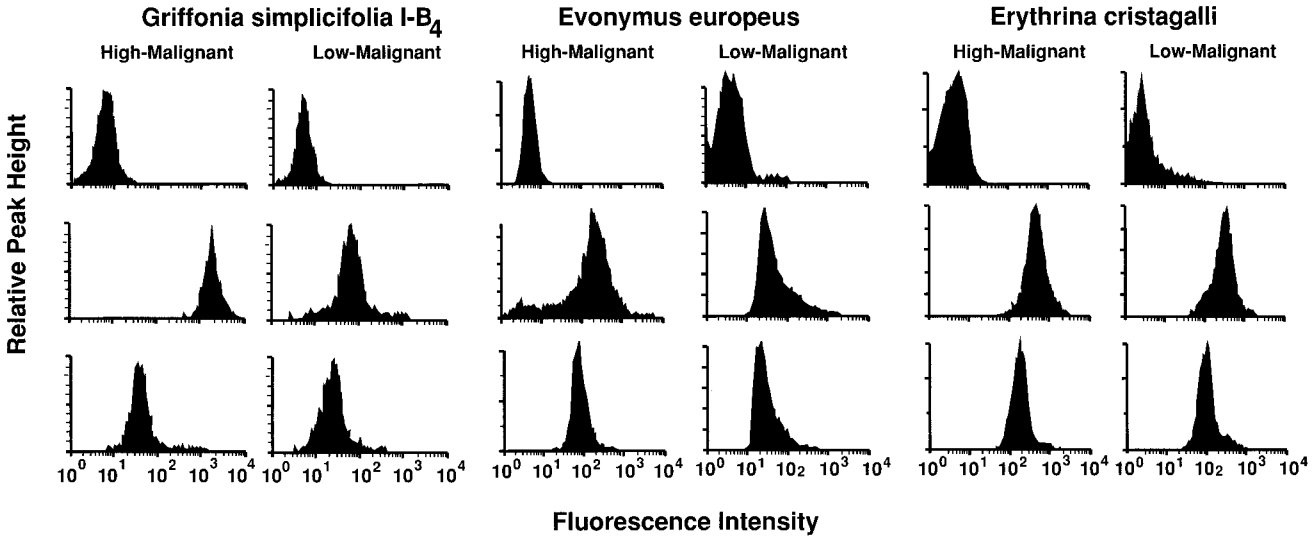

Figure 2. Flow cytometric analysis of high malignant (1.0) cells and low malignant (1.0/anti-B^r) cells after staining with GS I-B_4, Evonymus europea and *Erythina crystagalli* lectins. Lectin staining in the absence and presence of the respective inhibitors was carried out as described in the 'Materials and methods' section. With each lectin, the upper panel represents background staining (no lectin present); the middle panel represents total staining (lectin but no inhibitor) and the lower panel represents non-specific staining (lectin + inhibitor). Each cell line was examined on at least two occasions with similar results.

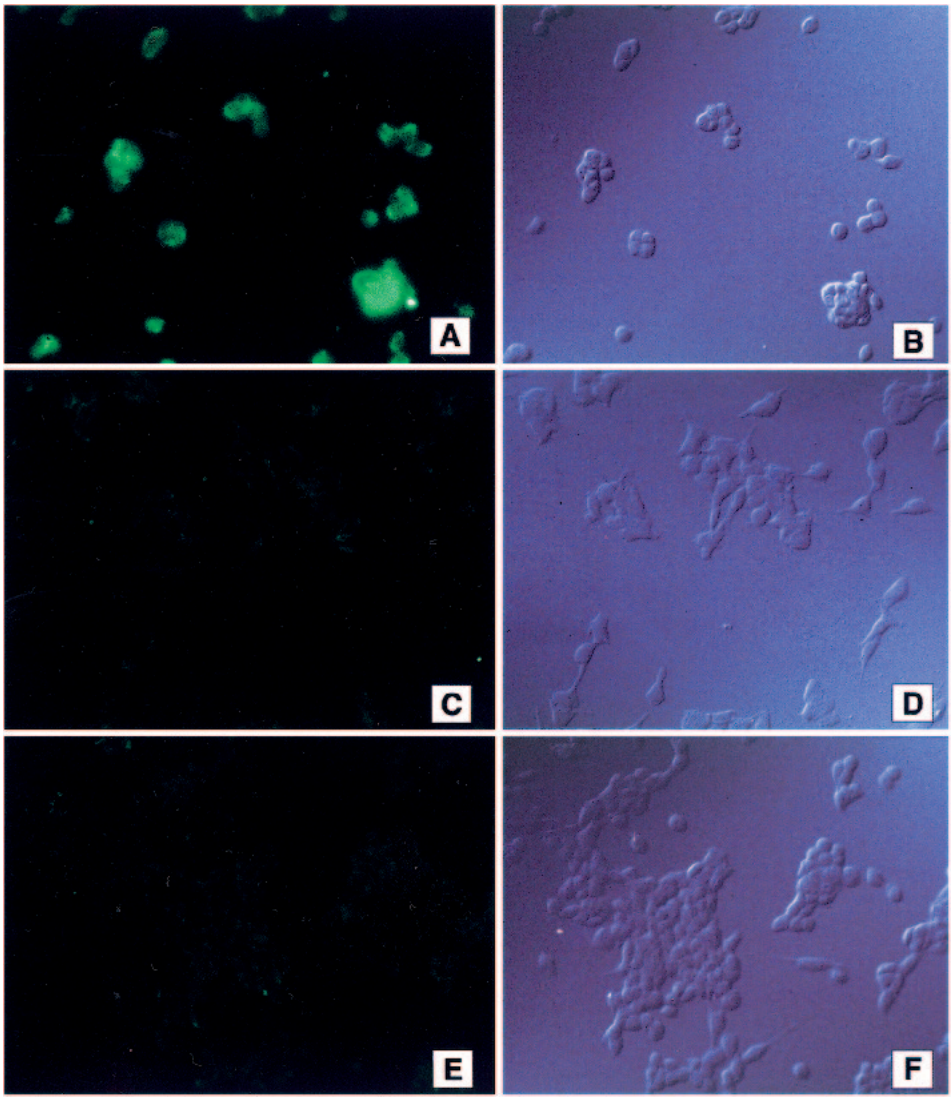

Figure 3. Effects of α-galactosidase treatment on binding of GS I-B_4 to high-malignant (1.0) sarcoma cells by indirect immunofluorescence. The upper (left) panel shows control 1.0 cells stained with GS I-B_4. The middle panel shows 1.0 cells following treatment with green coffee bean α-galactosidase and then stained with GS I-B_4. The lower panel shows control low-malignant (1.0/anti-B^r) cells stained with GS I-B_4. Right-side panels show corresponding phase-contrast photomicrographs.

α-Galactosidase activity in extracts of high and low malignant cells

Since it appeared from the above studies that only the terminal αGal was lacking on the low malignant cells, experiments were conducted to determine the reason for the lack of expression on these cells. Extracts were prepared from all six cell lines and assessed for α-galactosidase activity as described in the 'Materials and methods' section. The results (Table 2) indicate that although there were differences in total activity among the three pairs of lines, there was no significant difference between the high-malignant line and low-malignant line from any of the three tumors.

Table 2. α-galactosidase activity in extracts of high and low malignant murine fibrosarcoma cells.

Cell line	Enzyme activity (Δ O.D.$_{405}$ per 100 μg protein)
1.0	0.49 ± 0.01
1.0/anti-B^r	0.59 ± 0.01
1.1	0.49 ± 0.01
1.1/anti-B^r	0.50 ± 0.04
1.2	0.68 ± 0.02
1.2/anti-B^r	0.53 ± 0.02

α-Galactosidase activity was assessed as described in the 'Materials and methods' section using the p-nitrophenyl derivative of α-D-galactopyranoside as substrate. Values shown are means and standard deviations based on triplicate samples in a single experiment. Each cell line was assayed two separate times with similar results.

Galactosyl transferase activity in extracts of high and low malignant cells

Extracts were prepared from all six cell lines and assessed for galactosyl transferase activity as described in the Materials and methods section. The results (Table 3) show that

Table 3. Galactosyl transferase activity in extracts of high and low malignant murine fibrosarcoma cells.

Cell line	Enzyme activity (CPM of UDP-^{14}C-galactose transferred)
1.0	3005 ± 289
1.0/anti- B^r	142 ± 17
1.1	2332 ± 70
1.1/anti-B^r	0
1.2	2391 ± 144
1.2/anti-B^r	808 ± 43

Galactosyl transferase activity was assessed as described in the 'Materials and methods' section. Values shown are means and standard deviations based on triplicate samples in a single experiment. Each cell line was assayed two separate times with similar results. When cell extracts were omitted from the reaction mixture, less than 50 CPM of UDP-^{14}C-galactose was transferred. This values has not been subtracted from the values shown in the table. When asialoorosomucoid was omitted from the reaction mixture, 1505 ± 95 CPM was transferred. This values has been subtracted from the values shown in the table.

galactosyl transferase activity was much higher in each of the three high-malignant cell lines than in the corresponding low-malignant lines. The galactose acceptor used in these experiments, asialo-orosomucoid, is known to bind galactose in the α1-3 linkage [17]. It is possible, however, that not all of the ^{14}C-galactose bound to the acceptor was in this linkage. In order to demonstrate that at least some of the galactose bound to asialo-orosomucoid was in the α1-3 linkage, an extract prepared from the 1.0 (high-malignant) cell line was used to catalyze the transfer of ^{14}C-galactose to asialo-orosomucoid. Following transfer of the galactose to the asialo-orosomucoid, the galacose-bound acceptor was exposed to green coffee bean α-galactosidase. Following enzyme exposure, released radioactivity was separated from the acceptor and analyzed. A significant portion (i.e., approximately 16%) of the originally bound ^{14}C-galactose was released from the acceptor with this treatment. While this enzyme digestion procedure undoubtedly underestimates the amount of ^{14}C-galactose specifically linked to the subterminal galactose through the α1-3 linkage, it demonstrates that some of it is.

mRNA levels for α1-3 galactosyl transferase in high and low malignant cells

In a final series of experiments, RNA was prepared from 1.0 and 1.0/anti-B^r cells, separated by agarose-gel electrophoresis and blotted with radiolabeled (α1-3)GT cDNA insert as a probe for α1-3 galactosyl transferase mRNA. A single, indistinguishable band of approximately 4.15 kb in size was identified in both the high- and low-malignant cells (Figure 4), suggesting equal amounts of steady-state message for the α1-3-galactosyltransferase.

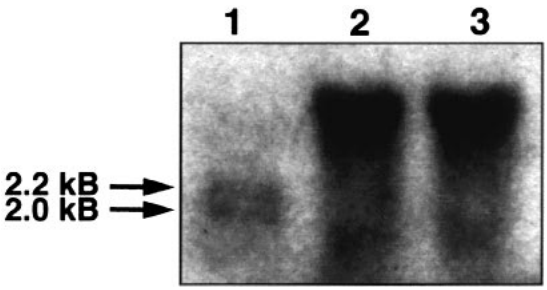

Figure 4. Northern blot analysis of RNA isolated from 1.0 and 1.0/anti-B^r cells. Total RNA (15 μg/lane) was fractionated on a 1.4% formaldehyde agarose gel and transferred to a nitrocellulose membrane. The α1-3 galactosyl transferase transcript (4.15 kb) was detected by hybridization with a ^{32}P-labeled (α1-3)GT cDNA insert.

Discussion

There is a strong correlation between cell surface expression of GS I-B_4-reactive carbohydrate moieties and malignant potential among murine sarcomas [2, 3, 5, 6]. This report presents evidence that the moiety responsible for lectin reactivity is the trisaccharide, Galα1-3Galβ1-4GlcNAc-. This is based on a correlation between reactivity of GS I-B_4-positive cells with a monoclonal antibody which is highly specific for the trisaccharide structure Galα1-3Galβ1-4GlcNAc- and lack of reactivity of GS I-B_4-negative cells with the same antibody. This monoclonal antibody does not react with a number of closely related sequences including Galβ1-4GlcNAc-bovine serum albumin and Galβ1-4GlcNAc-bovine thyroglobulin. Other related carbohydrate structures including Galβ1-4GlcNAc-, Galβ1-4Glc (lactose), Galα1-3Gal and Galα1-6Glc (melibiose) are also ineffective targets for the antibody [12]. Previous studies have identified this trisaccharide on other murine tumor cells [18–20], but its correlation with malignant potential had not been established.

Further studies reported here suggest that while the terminal αGal is missing from carbohydrate structures on the surface of low-malignant sarcoma cells (isolated originally from the same tumors as the high-malignant cells), the subterminal disaccharide Galβ1-4GlcNAc- is still present. This is based on the finding that the high-malignant cells (but not the low-malignant cells) also react with *Evonymus europaea* lectin while the high- and low-malignant cells react equally with *Erythina crystagalli* lectin. *Evonymus europaea* lectin is similar to GS I-B_4 in that it has specificity for galactose in the α1-3 linkage but unlike GS I-B_4, it does not react with galactose in the α1-4 linkage or with N-acetyl Lactosamine [21, 22]. Rather, *Evonymus europaea* has reactivity for αGal and for fucosyl residues in certain linkages [21]. *Erythina crystagalli* lectin, on the other hand, reacts with Gal β1-4GlcNAc – the most common acceptor for the terminal αGal [22]. The implication of these findings is that the loss or alteration of the single, terminal monosaccharide residue on the cell surface can have profound effects on biological function.

What accounts for the differential expression of the terminal αGal on the high- and low-malignant murine sarcoma cells is not fully understood. The data presented here demonstrate significant differences in the expression of

α-galactosyl transferase activity which correlates with the difference in terminal αGal expression. In contrast, there is no significant difference in galactosidase activity and (as indicated above) no apparent difference in expression of the subterminal disaccharide (Gal β1-4GlcNAc-) between the αGal expressing and non-expressing cells. Thus, it is concluded that the differential expression of α-galactosyl transferase activity is likely to be the major factor responsible for the differences in carbohydrate profile. At present, the molecular basis for the reduced level of α-galactosyl transferase activity in the low-malignant cells is not known. When steady-state mRNA levels for α1-3 galactosyl transferase were assessed in 1.0 and 1.0/anti-B^r cells, no differences were seen. This could suggest a point mutation or small deletion in the gene for this enzyme in the low malignant cells. The use of RT-PCR and nucleotide sequencing would help to determine if this were the case. Alternatively, a number of other explanations – post-transcriptional regulation, differences in message stability or alterations in the presence of essential co-factors or enzyme inhibitors – are possible. Additional studies will be needed to distinguish among these and other possibilities. These studies would seem to be warranted, however, since the relationship between terminal αGal expression and metastatic potential has been observed in a number of tumors, including some of human origin [10, 11].

How differential αGal expression on the surface of murine sarcoma cells influences tumor spread is not fully understood. Our past studies have shown that there is no significant difference between αGal-expressing and non-expressing sarcoma cells in their *in vitro* growth rates or saturation densities. In contrast, all of the αGal-expressing cells are more adhesive and more motile than the Gal α1-3-deficient cells [1, 3, 16, 23–25]. Cell-substrate adhesion and cell motility are important for tumor invasion and metastasis, and it may be that the expression of α-Gal on cell surface glycoproteins promotes these functions. It is of interest in this regard that laminin, which is a critical cell surface component in murine tumor metastasis [26], is glycosylated with Galα1-3Galβ1-4GlcNAc- [27]. Alternatively, it has been demonstrated that αGal-non-expressing (low-malignant) sarcoma cells are recongnized by a variety of immunological and inflammatory cells, while the Galα1-3-expressing (high-malignant) cells are not [28–30]. Thus, differences in *in vivo* behavior between αGal-expressing- and non-expressing cells may reflect differential interactions with cells that have cytotoxic activity.

Of interest, while the correlation between αGal expression and malignancy appears to be absolute among murine sarcomas, studies with murine melanoma cells provide different results. Specifically, it has been found that transfection of melanoma cells with α1,3 galactosyl transferase led to increased expression of αGal on the cell surface, and this concomitantly reduced lung colony formation by the cells after intravenous injection [31]. Subsequently it was shown that the transfected cells had a reduced level of surface sialic acid – not surprising, since galactose and sialic acid compete for the same subterminal acceptor [32]. It was also shown that capping the sub-terminal disaccharide with fucose rather than galactose had a similar effect (e.g., limiting sialic acid expression and reducing lung colony formation). On the basis of these results, it was suggested that failure to incorporate sialic acid (rather than a positive effect of galactose or fucose incorporation) into surface carbohydrates compromised capacity of the melanoma cells to carry out one or more functions that were critical to lung colony formation. This is consistent with past studies, which have shown that cell membrane sialyation is positively correlated with metastatic activity in a number of tumor models [33–35]. How sialic acid expression influences pro-metastatic events in melanoma cells is not fully understood. Sialic acid-containing structures are known to serve as adhesion ligands for leukocytes [36, 37] and expression of similar structures on some tumor cells has been shown to correlate with agressive behavior [38, 39]. Suffice to say in conclusion that the distinctly different results with murine sarcoma and melanoma cells attests to the fact that multiple mechanisms exist for cells to carry out the necessary steps in the metastatic process.

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References

1. Varani J, Orr W, Ward PA. Comparison of supopulations of tumor cells with altered migratory activity, attachment characteristics, enzyme levels and *in vivo* behavior. Eur J Cancer 1979a; 15: 585–92.
2. Varani J, Lovett EJ, Wicha M et al. Cell surface α-D-galactopyranosyl end groups: Use as a marker in the isolation of murine tumor cell lines with varying malignant potential. J Natl Cancer Inst 1983b; 71: 1281–6.
3. Varani J, Lovett EJ, McCoy JP et al. Differential expression of a laminin-like substance by high and low metastatic tumor cells. Am J Pathol 1983a; 111: 27–34.
4. Peters B, Goldstein IJ. The use of fluorescein-conjugated Bandeiraea simplicifolia B4 isolectin as a histochemical reagent for the detection of α-D-galactopyranosyl groups. Exp Cell Res 1979; 120: 321–4.
5. Grimstad IA, Bosnes V. Cell surface laminin-like molecules and α-D-galactopyranosyl end-groups of cloned strongly and weakly malignant murine fibrosarcoma cells. Int J Cancer 1987; 40: 505–10.
6. Castronova V, Foidart JM, Li Vecchi M et al. Human anti-alpha-galactosyl IgG reduces the lung colonization by murine MO4 cells. Invasion & Metastasis 1987; 7: 325–45.
7. Galili U, Shohet SB, Kobrin E. Man, apes and old world monkeys differ from other mammals in the expression of α-galactosyl epitopes on nucleated cells. J Biol Chem 1988; 263: 17755–62.
8. Towbin H, Rosenfelder G, Wieslander J. Circulating antibodies to mouse laminin in Chagas disease, American cutaneous leishmaniasis and normal individuals recognize terminal (α1-3) galactose epitopes. J Exp Med 1987; 166: 419–32.
9. Kagawa Y, Takasaki U, Utsumi J. Comparative study of asparagine-linked sugar chains of human interferon-β1 and recombinant human interferon-β-1 produced by three different mammalian cells. J Biol Chem 1988; 263: 17508–15.
10. Castronova V, Colin C, Parent B et al. Possible role of human natural anti-gal antibodies in the natural antitumor defense system. J Natl Cancer Inst 1989; 81: 212–6.

11. Petryniak J, Varani J, Ervin PR et al. Differential expression of glycoproteins containing α-D-galactosyl groups on human breast epithelial cells and MCF-7 breast carcinoma cells. Cancer Lett 1991; 60: 59–65.
12. Takagaki M, Knibbs RN, Roth J, Goldstein IJ. Monoclonal antibodies that recognize the trisaccharide epitope Gal α1-3 Gal β1-4 GlcNAc present on Ehrlich tumor cell membranes glycoproteins. Histochemistry 1993; 100: 139–47.
13. Galfre G, Howe SC, Milstein C et al. Antibodies to major histocompatability antigens produced by hybrid cell lines. Nature 1977; 266: 530–2.
14. McKearn TJ. Fusion of cells in an adherent monolayer. In Kennet RH, McKearn TJ, Bechtol KB (eds): Monoclonal Antibodies and Hybridomas: A New Dimension in Biological Analysis. New York: Plenum Press 1980; 368–9.
15. Larsen RD, Rajan VP, Ruff MM et al. Isolation of cDNA encoding a murine UDP-galactose: β-D-galactosyl-1,4-N-acetyl-D-glucosamine α-1-3-galactosyltransferase: Expression cloning by gene transfer. Proc Nat Acad Sci 1989; 86: 8227–31.
16. Varani J, Orr W, Ward PA. Hydrolytic enzyme activities, migratory activity and *in vivo* growth and metastatic potential for recent tumor isolates. Cancer Res 1979b; 39: 2376–80.
17. Rademacher TW, Parekh RB, Dwek RA. Glycobiology. Ann Rev Biochem 1988; 57: 785–838.
18. Eckhardt AE, Goldstein IJ. Isolation and characterization of a family of α-D-galactosyl-containing glycopeptides from Ehrlich ascites tumor cells. Biochemistry 1983; 22: 5290–8.
19. Cummings RD, Kornfeld S. The distribution of repeating [Galβ1,4GlcNAcβ1,3] on mouse lymphoma cell line BM5147 and PHAR2.1. J Biol Chem 1984; 259: 6253–60.
20. Santer UV, DeSantis R, Hard KJ et al. N-linked oligosaccharide changes with oncogenic transformation require sialyation of multiantennae. Eur J Biochem 1989; 181: 249–60.
21. Petryniak J, Goldstein IJ. Immunochemical studies on the interaction between synthetic glycoconjugates and α-L-fucosyl binding lectins. Biochemistry 1986; 25: 2829–38.
22. Adar R, Richardson M, Lis H et al. The amino acid sequence of Erythina corollodendron lectin and its homology with other legume lectins. FEBS Lett 1989; 257: 81–5.
23. Varani J. Lovett EJ, Elgebaly S et al. *In vitro* and *in vivo* adherence of tumor cell variants correlated with tumor formation. Am J Path 1980; 101: 345–52.
24. Varani J, Lovett EJ. Phenotypic stability of tumor cells *in vitro* and *in vivo*. J Natl Cancer Inst 1982; 68: 957–62.
25. Varani J, Fligiel SEG, Perone P. Directional motility in malignant murine tumor cells. Int J Cancer 1985; 35: 559–64.
26. Malinoff HL, Mccoy JP, Varani J et al. Metastatic potential of murine fibrosarcoma cells is influenced by cell surface laminin. Int J Cancer 1984; 33: 651–5.
27. Knibbs RN, Perini F, Goldstein IJ. Structure of the major concanavalin A reactive oligosaccharides of the extracellular matrix component laminin. Biochemistry 1989; 28: 6379–92.
28. Lovett EJ, III, Dickinson RW, Varani J. Metastatic variants from a methylchonanthrene induced syngeneic murine fibrosarcome produce immunosuppression proportional to the metastatic potential of the variant. In Lapis K, Jeney A, Price MR (eds): Tumor Progression and Markers, Amsterdam: Kugler 1982; 145–53.
29. Laybourn KA, Abruzzo LV, Varani J. *In vitro* and *in vivo* interaction between murine fibrosarcoma cells and natural killer cells. Cancer Res 1986; 46: 3407–12.
30. Riser BL, Laybourn KA, Varani J. Treatment of mice with antibody to asialo-GM, or poly I:C: Effects on metastasis dissociable from modulation of macrophage anti-tumor activity. Nat Imm Cell Growth Regulation 1988; 7: 305–15.
31. Gorelik D, Duty L, Anaraki F et al. Alterations of cell surface carbohydrates and inhibition of metastatic property of murine melanomas by α1,3 galactosyltransferase gene transfection. Cancer Res 1995; 55: 4168–73.
32. Gorelik E, Xu F, Henion T et al. Reduction of metastatic properties of Bl6 melanoma cells expressing terminal fucose a-2-galactose after a1,2-fucosyltransferase cDNA transfection. Cancer Res 1997; 57: 332–6.
33. Yogeeswaran G, Salk P. Metastatic potential is positively correlated with cell surface sialyation of cultured murine tumor cell lines. Science (Washington DC) 1981; 212: 1514–6.
34. Dennis J, Donaghue T, Kerbel R. Membrane associated alterations detected in poorly tumorigenic lectin-resistant variant sublines of a highly malignant and metastatic murine tumor. J Nat Cancer Inst 1981; 66: 129–39.
35. Altevogt P, Fogel M, Chengsong-Popov R et al. Different patterns of lectin binding and cell surface sialyation detected on related high and low metastatic tumor lines. Cancer Res 1983; 43: 5138–44.
36. Lowe JB, Stoolman LM, Nair RP et al. ELAM-1 dependent cell adhesion to vascular endothelium determined by a transfected human fucosyl trasferase cDNA. Cell 1990; 63: 475–84.
37. Lasky LA, Singer MS, Dowbenke D. An endothelial cell ligand for L-selectin is a novel mucin-like molecule. Cell 1992; 69: 927–38.
38. Matsushita Y, Nakamori S, Irimura T. Human colon carcinoma cells with increased invasion capacity obtained by selection for sialyldimeric Lex antigen. Exp Cell Res 1991; 196: 20–5.
39. Nakamori S, Kameyama M, Kabuto T et al. Increased expression of sialyl Lewis x antigen correlates with poor survival in patients with colorectal cancer: Clinicopathological and immunochemical study. Cancer Res 1993; 53: 3632–7.
40. Kabat E, Leskowitz S. Immunochemical studies on blood groups. XVII. Structural units involved in blood groups A and B specificity. J Amer Chem Soc 1955; 7: 5159–64.
41. Chirgwin JM, Przybyla AE, MacDonald RJ et al. Isolation of biologically-active ribonucleic acid from sources enriched in ribonuclease. Biochemistry 1979; 24: 5294–9.