H Blood Group Antigen Carried by CD44V Modulates Tumorigenicity of Rat Colon Carcinoma Cells

N. Labarrière, J. P. Plau, C. Otry, M. Denis, P. Lustenberger, K. Meflah, and J. Le Pendu

INSERM CJF 90-11, Department of Medical Biochemistry, Institut de Biologie, 9 Quai Moncousu, F-44035 Nantes Cedex 01, France

ABSTRACT

Expression of carbohydrate ABH blood group antigens is onco-developentially regulated and their presence on tumor cells constitutes a prognostic factor. However, it is not clear whether they directly affect tumor behavior. Using a rat model of colon carcinoma, we previously observed an association between the presence of H blood group antigens and tumorigenicity in syngeneic animals. In the present study, we show by immunoprecipitation experiments that cell surface H blood group antigens of a highly tumorigenic clone (PROb) are essentially carried by splice variants of the CD44 molecule containing exon V6. PROb cells were then transfected with an antisense fragment of the gene coding for a rat α(1–2)fucosyltransferase. This enzyme allows synthesis of H antigens from various β-galactoside precursors. Transfected subclones of PROb cells were obtained which had significantly decreased enzymatic activity and H antigenic cell surface levels. In contrast, no such changes were observed in control cells transfected with either the empty vector or with a sense fragment of the gene. Compared to controls, the antisense-transfected cells were far less tumorigenic in syngeneic animals. These results show that H blood group antigens at the surface of PROb colon carcinoma cells contribute to tumor progression. The presence of the fucosylated structures on CD44 could modulate the functions of this adhesion molecule.

INTRODUCTION

Aberrant glycosylation is an essential feature of tumor cells (1). The most common alterations, in the case of carcinoma are changes in ABH and Lewis related histo-blood group antigens. Synthesis of these antigens is regulated during development, and alterations of their normal expression on adult cells occur in tumors. Especially in the case of colorectal carcinomas, an intense expression close to that of the fetus has been evidenced, whereas normal adult distal colonic epithelial cells are devoid of such antigens (2–4).

These carbohydrate structures are built up by sequential addition, catalyzed by specific glycosyltransferases, of sugar residues onto a few oligosaccharidic precursor structures. H blood group antigens are formed by substitution of the terminal β-galactosyl residue of the various precursors by a fucose linked in α(1–2) thus masking the antigenicity of the precursors. The resulting H-active oligosaccharides can be further substituted on the galactose by either an N-acetylgalactosamine or a galactose linked in α(1–3) to give the A and B antigens, respectively, again masking the H antigens (5). They can also be substituted by a fucose linked in α(1–4) or α(1–3) on the penultimate N-acetylgalactosamine of the type 1 and 2 precursors to give the Lea and Leb antigens, respectively. The key enzyme in this metabolic pathway is the α(1–2)fucosyltransferase (EC 2.4.1.69) responsible for synthesizing H antigens by addition of L-fucose to the terminal galactosyl residues of blood group type oligosaccharides on glycoproteins or glycolipids (6).

A high level of H/Lea/Leb antigens recognized by a monoclonal antibody was shown to correlate with a lower survival of patients with lung carcinoma (7). Very recently, the same observation was made for colorectal carcinoma using another antibody of similar specificity (8).

We previously studied the expression of blood group carbohydrate antigens on a series of rat colon cancer clones originating from the same parental cell line and differing by their tumorigenic potential in the syngeneic host. We observed an association between the tumorigenicity of the clones and the presence of cell surface H blood group antigen or α(1–2)fucosyltransferase activity (9). However, this association could have been fortuitous. Thus, the purpose of the present study was to investigate more directly the role of fucosylated structures on the tumorigenic potential of colon tumor cells by specifically modulating α(1–2)fucosyltransferase activity using an antisense fragment of the corresponding gene. Recently, two partial sequences coding for two distinct rat α(1–2)fucosyltransferases (FTA and FTB) have been cloned in our laboratory (10). Both FTA and FTB genes are highly expressed in rat normal colon and in PROb cells. Transient transfection assays were performed with the partial sequences in antisense in PROb cells. In FTA and FTB antisense-transfected cells, 20 and 80% decreases in α(1–2)fucosyltransferase activity were, respectively, obtained (10). Therefore, the FTB complementary DNA fragment was chosen to produce stable transfectants and to investigate the implication of α(1–2)fucosyltransferase and H structures in the tumorigenicity of the rat colon carcinoma cells.

MATERIALS AND METHODS

Cell Culture. PROb rat colon adenocarcinoma (obtained from the European Collection of Animal Cell Culture, Salisbury, United Kingdom) is a clone derived from a dimethylyhydrazine-induced cell line (DHD12). The clone originates from a subline (TR) selected for its relatively high resistance to trypsin detachment and was chosen for its high tumorigenicity in syngeneic BDIX rats (11). Cells were cultured in complete medium (RPMI 1640, 5% fetal calf serum, 2 mM L-glutamine, 100 units/ml penicillin, and 100 µg/ml streptomycin; Gibco BRL, Cergy-Pontoise, France). They were subcultured by 0.025% trypsin in 0.02% EDTA (Gibco) for 10 min and replated at a 1/5 split. Stable transfectants were cultured in the same medium supplemented with 0.4 mg/ml of G418 (Sigma Chemical Co., St. Louis, MO). Cells were checked on a regular basis for Mycoplasma contamination.

Lectins and Antibodies. The lectins used were peanut agglutinin recognizing terminal β-D-galactosides, and UEA-I which recognizes the H-type 2 and Y oligosaccharides. These lectins were FITC-labeled and purchased from Sigma. Anti-Leb monoclonal antibody Leb2 was kindly provided by R. H. Fraser (Glasgow, United Kingdom). This IgM antibody reacts equally well with the Leb, H type 1, 3, and 4 structures but only weakly with H type 2 (12). In this study it has been used as an anti-H since the Leb structure is not present on the rat cells used, due to the absence of the Lewis α(1–4)fucosyltransferase (data not shown). Anti-H monoclonal antibody 1E3 is a kind gift from Dr. T. Nakajima (Gunma University, Gunma, Japan). It is an IgM that reacts with H types 1, 2, 3, and 4 equally well (13). Antibody 1.1AASM was obtained through the kindness of Professor P. Herrlich (Karlsruhe, Germany). It is an IgG and recognizes an epitope lying within the sequence of exon V6 of the CD44 molecule (14). Antibodies 3C9 and ME361, obtained from Dr. H. Clausen (Copenhagen, Denmark) and Dr. Z. Stepniowski (Philadelphia, PA), an anti-T blood group antigen, and an anti-GD2,

Received 7/11/94; accepted 10/4/94.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This work was supported by Grant 6871 from the Association pour la Recherche sur le Cancer (ARC, France) and by the Ligue Contre le Cancer (Loire-Atlantique, France).

2 To whom requests for reprints should be addressed.

The abbreviations used are: UEA-I, Ulex europaeus agglutinin; FITC, fluorescein isothiocyanate; PBS, phosphate-buffered saline; BSA, bovine serum albumin.

6275
respectively, were used as negative controls for IgM and IgG. FITC-labeled anti-mouse antibody was purchased from Sigma.

Construction of Antisense Expression Vector PSG5neo. All restriction enzymes and buffers were purchased from Promega (Madison, WI). pNeo plasmid (Pharmacia, Piscataway, NJ) was digested with BamHI and HindIII and the fragments were blunt-ended by treatment with Klenow enzyme. PSG5 plasmid (Stratagene, La Jolla, CA) was linearized by digestion with XbaI and blunt-ended. After phenol-chloroform extraction and ethanol precipitation, a ligation was performed using 200 ng of each product with a ligation kit (Pharmacia). Following transfection in XL1-Blue E. coli competent cells (Stratagene), bacteria were split on agar containing ampicillin (20 μg/ml) and G418 (8 μg/ml). The rat α1(2)-fucosyltransferase FTB complementary DNA fragment (977 base pairs) was inserted in the unique EcoRI cloning site. Orientation of the inserts was obtained by sequencing.

Cell Transfection and Isolation of Stable Transfectants. Stable transfectants were produced by transfection of 20 μg of recombinant plasmid in PROB cells grown in 60-mm Petri dishes, using lipofectin, as described by the supplier (Gibco BRL). After 24 h and every 2 days thereafter, the medium was replaced with fresh medium containing 0.4 mg/ml of neomycin analog G418 to select resistant clones. After 2 weeks, cell colonies were isolated. One cell clone was expanded as described above. The cells were rinsed with ice-cold PBS, pH 7.2, and recovered by scraping. After the cells were washed with ice-cold PBS, they were solubilized in 50 mM potassium phosphate, pH 6.0, containing 1% (v/v) Triton X-100. Following centrifugation at 13,000 × g for 10 min, the supernatant was collected and used as crude enzyme preparation. Protein concentration was determined using bicinchoninic acid obtained from Pierce (Rockford, IL). The reaction mixture contained: 20 μM GDP-[3H]fucose (23 μCi/mmol; NEN Chemical Center, Dreieichenheim, Germany), 46 mM phenyl-β-D-galactopyranoside, 10 mM L-fucose, 7.7 mM MgCl2, 1.9 mM ATP, and 50 μg of protein extract. After incubation at 37°C for 3 h, the reaction mixture was quenched with 5 μl of distilled water and applied to a freshly conditioned C18-Sep Pak cartridge (Waters-Millipore) as described by Palci et al. (15). The cartridge was washed with 20 ml of water. Radiolabeled product ([3H]fucosylphenyl-β-D-galactopyranoside) was then eluted with 5 ml methanol and counted in 10 ml scintillation liquid Ready Safe (Beckman, Palo Alto, CA). Background levels of radioactivity were measured as follows. A control sample, consisting of 25 μg of proteins from cells grown in 60-mm Petri dishes, using lipofectin, as described by the supplier (Gibco BRL), was processed as described above. The remaining part of the sample was then used for immunoprecipitation. Using the anti-H antibody, 20 μg of protein extract was incubated with 20 μl of protein A-coupled beads (Sigma). Then, 150 μg of proteins were incubated at 4°C with monoclonal antibodies for 16 h. The mixtures were next incubated for 2 h at 4°C with 50 μl of anti-immunoglobulin-coupled agarose beads preadsorbed with 3% defatted milk in PBS. Mouse anti-IgM or anti-IgG-coupled beads were used according to the class of the primary antibody. Beads were washed 4 times with PBS containing 0.5% Triton X-100 and bound proteins were eluted by boiling in electrophoresis sample buffer containing 5% β-mercaptoethanol and submitted to electrophoresis in 5–15% gradient polyacrylamide gels. Separated proteins were then submitted to Western blotting as described above.

Animals. Inbred BDIX rats were purchased from Iffa-Credo (L’Abresle, France) and housed and bred under standard conditions in our laboratory. Animals two to three months old were used.

Tumorigenicity Assays. Confluent cells were trypsinized and injected s.c. in the flank of syngeneic BDIX rats (5 or 10 rats/group). BDIX rats received 106 cells suspended in 1 ml of RPMI supplemented with 5% fetal calf serum. Tumors were measured weekly with calipers. After 60 days, rats were killed under anesthesia with Rompun-Ketalar. Induction of peritoneal carcinomatosis was also performed with these cells. Two-month-old BDIX rats (5 rats/group) were given injections of 2 × 105 cells suspended in 1 ml of RPMI.

Immunohistochemistry. Pieces of s.c. tumors were fixed in ethanol:acetic acid (95:5) for 48 h and paraffin embedded. Sections (4 μm) were dehydrated in graded ethanol and washed in PBS. They were then incubated in 0.3% methanol/H2O2 for 20 min to block endogenous peroxidase and washed for 5 min in PBS. The tumor sections were then covered with PBS/BSA 3% for 20 min at room temperature in a humidified atmosphere. After sections were washed in PBS, they were covered with the primary anti-H antibody LM 13/276 diluted in 1% PBS/BSA and left at room temperature for 1 h. Sections were then rinsed twice with PBS and covered with biotinylated secondary antibody (Vector Laboratories, Burlingame, CA) for 1 h at room temperature. After the sections were washed in PBS, they were covered with peroxidase-conjugated avidin (Vector) for 45 min, washed with PBS, and covered with 3,3’-diaminobenzidine tetrahydrochloride solution 0.05% diaminobenzidine tetrahydrochloride and 0.01% H2O2 in Tris- HCl, pH 7.6) for 15 min. Counterstaining was performed with 1% Harris hematoxylin.

RESULTS

H Carbohydrate Antigens of PROB Cells Are Carried by Variants of CD44. In order to define the profile of glycoproteins carrying H antigenic determinants, total cell extracts were analyzed by immunoprecipitation followed by Western blotting. Using the anti-H antibodies, a major band at about M, 200,000 and some minor bands of lower molecular weight were immunostained (Fig. 1). These results showed that fucosylated structures were unexpectedly presented by a very restricted set of glycoproteins.

The restricted set of bands decorated by the anti-H antibodies was reminiscent of that observed by others with variants of the CD44 molecule (16, 17). In addition this glycoprotein displays many potential O-glycosylation sites in its extracellular domain (18, 19). We therefore decided to examine the possibility that the protein bands revealed by the antibody could be CD44 variants. This was performed using monoclonal antibody 1.1ASML. The epitope recognized by this antibody has been defined by Günthert et al. (14) on the product of alternatively spliced exon v6 of CD44 variants. The material immunoprecipitated with antibody 1.1ASML was thus immunostained with the anti-H antibody or with antibody 1.1ASML. The same bands were labeled by the two reagents. The converse was also true since material immunoprecipitated with the anti-H antibody reacted similarly with the anti-H and the anti-CD44v6 reagents, although less material was precipitated with the anti-H than with the...
Obtainment of Stable Transfectants. PROb cells were transfected either with PSGNeo or PSGNeo-containing inserts in sense or antisense orientations. Three clones (C1, C2, C3) containing PSG5Neo without inserts were isolated and used as controls in all experiments. Six clones (S1–S6) containing PSG5Neo with the partial sequence coding for the rat α(1–2)fucosyltransferase in sense orientation were obtained and tested for their enzymatic activity. Two of them were used in in vivo assays to control that this insertion did not alter their tumorigenicity. Finally, 4 clones (AS1, AS2, AS3, and AS4) containing the 1-kilobase fragment in an antisense direction were isolated. Their morphology was similar to that of control cells. Cell growth was measured in vitro by counting and proliferation curves were compared. No significant differences were observed among the various clones (data not shown).

α(1–2)Fucosyltransferase Activity. Enzyme activity was measured in all clones. It catalyzes the addition of L-fucose to position 2 of terminal nonreducing β-galactoside residues. No significant differences could be observed between PROb cells, controls or sense clones. In contrast, all antisense clones exhibited a reduced α(1–2)fucosyltransferase activity. AS1 presented a slight but nonsignificant decrease (about 25%) of enzymatic activity. The decrease in α(1–2)fucosyltransferase was about 30% for AS3 and AS4 clones and reached up to 55% for the AS2 clone (Fig. 2). Enzymatic activity was measured after 10 weeks of culture in G418 medium and the phenotype remained stable. Enzymatic kinetic parameters evaluated in a PSG5Neo control and the AS2 clone are presented in Table 1. AS2 presented a decreased V_{max} but the same affinity for phenyl-β-D-galactopyranoside, indicating that transfection affected enzyme expression without affecting enzyme affinity.

Flow Cytometric and Western Blotting Analyses. To determine whether the decrease in α(1–2)fucosyltransferase activity was associated with a change in fucosylated membrane glycoconjugates, a flow cytometric analysis was performed with fluorescent lectins and an anti-H antibody. PROb and control groups expressed similar amounts of H antigen. However, a dramatic reduction in the expression of H antigen was observed for AS2 and AS3 whether tested by anti-CD44v6 (Fig. 1A). The specificity of these reactions is assessed by the lack of labeling when using irrelevant antibodies either in the immunoprecipitation or in the Western blotting steps. These results show that the glycoproteins bound by the anti-H and anti-CD44v6 antibodies are the same and hence that the H carbohydrate structures on PROb cells are essentially borne by variants of the CD44 molecule. Furthermore, two successive absorptions of PROb CD44v6 with antibody 1.1ASML removed virtually all CD44v6-reactive material as shown that the glycoproteins bound by the anti-H and anti-CD44v6 antibodies are the same and hence that the H carbohydrate structures on PROb cells are essentially borne by variants of the CD44 molecule. Furthermore, two successive absorptions of PROb CD44v6 with antibody 1.1ASML removed virtually all CD44v6-reactive material as well as the anti-H-reactive material (Fig. 1B), thus showing that CD44v6 is the only glycoprotein detected by Western blot that carries H carbohydrate antigens on PROb cells.

The type of H antigenic determinants cannot be defined at present since antibody 1E3 binds equally well to H types 1, 2, 3, and 4 (13). In addition to glycoproteins, H antigens could be carried by glycolipids. However, only trace amounts of a glycolipid that could correspond to H-triglycosylceramide could be detected on PROb cells. Thus, H antigenic determinants of PROb cells are essentially carried by CD44v6.

Table 1. Kinetic parameters of α(1→2)fucosyltransferase activity of PROb, control, and AS2 clones, determined toward the acceptor phenyl-β-D-galactopyranoside.

<table>
<thead>
<tr>
<th>Cell type</th>
<th>V_{max} (nmol/h/mg)</th>
<th>K_{m} (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PROb</td>
<td>2.2</td>
<td>7.2</td>
</tr>
<tr>
<td>Control</td>
<td>2.1</td>
<td>7.4</td>
</tr>
<tr>
<td>AS2</td>
<td>1.0</td>
<td>7.6</td>
</tr>
</tbody>
</table>

* A. Ménoret et al., submitted for publication.
H BLOOD GROUP ON CD44 AND TUMORIGENICITY

Fig. 3. Cytofluorimetric analysis on control and antisense clones. Binding of the lectin UEA-I which binds H-type 2 residues (A); of monoclonal anti-H type 1, 3, and 4 LM 137/276 (B); and of peanut agglutinin which binds β-galactoside precursors (C); D, negative controls in absence or in presence of secondary anti-IgG FITC.

UEA-I (Fig. 3A) or by the LM137/276 anti-H antibody (Fig. 3B), this reduction being more important for AS2. The lectin peanut agglutinin was used to detect the levels of β-galactoside precursors expressed on cell membrane in control, AS2, and AS3 clones. Fig. 3C shows a good correlation between the decrease of H antigen expression and the increase in precursor expression, confirming the deficient fucosylation of the antisense-transfected clones. In accordance with these results, by Western blotting, anti-H-labeled glycoproteins were poorly detected on antisense clones, particularly in the case of clone AS2 (Fig. 4).

Antisense Transfectants Are Less Tumorigenic. PROb, controls (C1 and S1), AS2, and AS3 were injected s.c. into syngeneic BDIX rats and tumor growth was followed for 60 days (Fig. 5). Tumorigenic potentials of antisense clones have been compared either to control clones or to clones transfected with PSG5 plasmid containing a “sense” fragment. These controls have been constructed to ensure that the transfection process or the possible expression of peptides would not disturb the ability of cells to grow in vivo with particular respect to the immune response of the host. Tumorigenic behaviors of transfected control clones were similar to that of PROb nontransfected cells. In contrast, AS2 and AS3 tumor growth was delayed. Their growth began 15 to 20 days after the control groups. Development of AS3 tumor was clearly delayed but tumor volumes reached those of control tumors after 60 days (not shown). On the contrary, AS2 tumors did not reach controls within 60 days, their volumes or weights remaining smaller.

Tumors were assayed for their α(1–2)fucosyltransferase activity after 60 days to ensure that antisense plasmid was still functional, and important differences were observed between controls and antisense clones (data not shown). Furthermore, tumor sections were assayed for the expression of fucosylated structures with an anti-H specific antibody. A strong staining was observed on peripheral PROb and control tumor cells (Fig. 6); no staining at all was observed with a control IgM antibody. In contrast, this staining was weaker for the AS3 tumors and barely detectable on AS2 tumor sections, indicating that the antisense fragment remained expressed in vivo. Histological
vascular endothelium, thereby facilitating metastatic dissemination as cell adhesion proteins, which play crucial roles in the initial stages of correlation with the invasive capacities of tumor cells (20—23). The fucosylated. They are expressed by many tumor cell types (but not by PROb cells) and thus could mediate adhesion of cancer cells to leukocyte recruitment and extravasation during inflammation, recognize the sialyl-Le^a and sialyl-Le^b oligosaccharides (24). These carbohydrates of the histo-blood group family are both sialylated and nized the sialyl-Le^a and sialyl@Le^a oligosaccharides (24). These carbo

**DISCUSSION**

Altered fucosylation of cancer cells has been observed many times and in experimental models, increased fucosylation was shown to correlate with the invasive capacities of tumor cells (20—23). The recent characterization of carbohydrate structures recognized by selectins may well explain in part these earlier findings. Indeed, these cell adhesion proteins, which play crucial roles in the initial stages of leukocyte recruitment and extravasation during inflammation, recognize the sialyl-Le^a and sialyl-Le^b oligosaccharides (24). These carbohydrates of the histo-blood group family are both sialylated and fucosylated. They are expressed by many tumor cell types (but not by PROb cells) and thus could mediate adhesion of cancer cells to vascular endothelium, thereby facilitating metastatic dissemination as recently suggested (25, 26). However, other fucosylated structures exist, not recognized by selectins yet characterized, which expression is developmentally regulated and altered during tumorigenesis. Very few studies directly addressed the biological role of such carbohydrates. However, Miyake and Hakomori (27) reported that a monoclonal antibody selected for its ability to inhibit cell motility recognized the H blood group antigen irrespective of the subjacent structure. Expression of H/Le^a/Le^b antigens was later shown to correlate with a lower survival of patients with either lung or colon carcinomas (7, 8). In order to more directly assess the function of blood group H carbohydrate structures in tumor progression, we specifically inhibited the synthesis of H antigens by rat colon tumor cells using an antisense fragment of the α(1—2)fucosyltransferase responsible for their synthesis.

Since tumor cell lines are quite heterogeneous, we decided to transfect the antisense fragment in already cloned cells. This was done in order to minimize the risk of selecting out clones with a decreased expression of the enzymatic activity due to heterogeneity of the parental cell line rather than to the transfected antisense fragment. PROb is a highly tumorigenic clone issued from a dimethylhydrazine-induced colon adenocarcinoma (11). Only early passages of the clones were used in this study. These cells present H type 2 structures at their cell surface as shown by the reactivity of the UEA-I lectin and of anti-H type 2 specific monoclonal antibodies (not shown). In addition, they present H type 1 and/or 3 structures as assessed by the reactivity of antibody LM 137/276. Transfection with an antisense fragment of a rat α(1—2)fucosyltransferase resulted in a decrease of the enzymatic activity of all the subclones isolated, but in none of the control transfected subclones, establishing that the decrease was specifically mediated by expression of the antisense fragment. The AS2 and AS3 clones, which showed the most important drop in enzymatic activity, were chosen for subsequent studies. It appeared that their surface examination also revealed that the AS2 and AS3 tumors differed from the parental PROb and control tumors. The latter were poorly differentiated with pseudoglandular structures. In AS3 and most obviously in AS2 tumors, the overall aspect was more heterogeneous with large undifferentiated areas.

In order to confirm the decrease of tumorigenicity of the antisense transfectants observed after s.c. injections, PROb, controls (C1, and S1), AS2, and AS3 clones were injected i.p. into syngeneic BDIX rats to induce peritoneal carcinomatosis. Survival of rats bearing PROb and control clones were not different; survival medians were, respectively, 34, 34, and 37 days. The survival of rats bearing the AS3 clone were slightly higher (survival median, 49 days). Survival times of the AS2 group were much higher with a survival median of 69 days and one animal surviving over 120 days without any sign of disease, again showing a direct relationship between expression of the H blood group antigen and the aggressiveness of the tumor cells (Fig. 7).
expression of H antigens was greatly diminished and that conversely, their expression of \( \beta \)-galactoside precursors was increased as expected. It was nevertheless surprising that a decrease of enzymatic activity of about 50\% led to such a dramatic change in cell surface glycosylation. This would suggest that in PROb cells, the amount of fucosyltransferase is a rate-limiting factor of this glycosylation step. Regardless, clones AS2 and AS3 proved less tumorigenic than parental and control clones, this effect being more pronounced for the AS2 clone, which also had the weakest enzymatic activity. Thus, in this experimental model, there exists a direct relationship between expression of the H antigen and the tumorigenic potential of the cells. An effect on the metastatic potential is more difficult to establish since PROb cells, when injected s.c., form metastases to the axillary lymph nodes and to the lungs, only in less than 50\% of animals. Since the AS2 and AS3 clones formed only slow growing tumors they did not form detectable metastases during the time of this study (data not shown).

Macrophage C-type lectins recognizing terminal \( \beta \)-galactosides unmasked by the loss of either sialic acid or fucose residues have been described (28, 29). Recently, it was shown that natural killer cells possess a unique C-type lectin which could be involved in the recognition of target cells (30). It would thus be possible that a deficient fucosylation would render PROb cells recognizable by such a lectin of either macrophages or natural killer effectors. Alternatively, the function of some membrane glycoproteins of tumor cells could be affected directly by their degree of fucosylation. In this context, it is noteworthy that the H antigens of PROb cells are borne by variants of the CD44 molecule. CD44 is detected on many cell types including lymphocytes, fibroblasts, and epithelial cells. It has been shown to play a role in lymphocyte activation, cell-cell adhesion, and cell-matrix interactions. Various isoforms of this molecule have been described which consist of posttranslationally modified products of primary (standard) CD44 or alternatively spliced (variant) CD44 transcripts. These variant proteins (CD44v) differ from the standard.

Fig. 6. Immunostaining of paraffin-embedded 45-day tumor sections with LM 173/276 anti-H antibody as described in "Materials and Methods." A, PROb; B, PSG5 control (C1); C, AS3; D, AS2. Bars, 20 \( \mu \)m.
form by the addition of a highly variable region, located just outside the cell membrane; 10 different exons are available for splice variation (reviewed in Ref. 31). Expression of CD44v molecules confers metastatic ability to pancreatic rat carcinoma cells (14). Recently, Rudy et al. (16) observed that expression of CD44 variants sharing the region around antibody 1.1ASML epitope was sufficient to confer metastatic behavior. The variant portion of the smallest CD44v was composed of only 2 exons, namely v6 and v7. In addition, strong expression of variants containing exon v6 sequences was observed in the more advanced stages of colon carcinoma and in metastasis (17, 32, 33). CD44 is also known as a heavily glycosylated protein that confers metastatic potential to rat carcinoma cells. Cell, 65: 13—24, 1991.

Acknowledgments

The authors thank Dr. R. H. Fraser, Dr. T. Nakajima, and Dr. P. Herrlich for their generous gifts of antibodies.

References


Form by the addition of a highly variable region, located just outside the cell membrane; 10 different exons are available for splice variation (reviewed in Ref. 31). Expression of CD44v molecules confers metastatic ability to pancreatic rat carcinoma cells (14). Recently, Rudy et al. (16) observed that expression of CD44 variants sharing the region around antibody 1.1ASML epitope was sufficient to confer metastatic behavior. The variant portion of the smallest CD44v was composed of only 2 exons, namely v6 and v7. In addition, strong expression of variants containing exon v6 sequences was observed in the more advanced stages of colon carcinoma and in metastasis (17, 32, 33). CD44 is also known as a heavily glycosylated protein that confers metastatic potential to rat carcinoma cells. Cell, 65: 13—24, 1991.

Acknowledgments

The authors thank Dr. R. H. Fraser, Dr. T. Nakajima, and Dr. P. Herrlich for their generous gifts of antibodies.
H Blood Group Antigen Carried by CD44V Modulates Tumorigenicity of Rat Colon Carcinoma Cells
