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Altered Glycosylation of α(s)β1 Integrins From Rat Colon Carcinoma Cells Decreases Their Interaction With Fibronectin

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Abstract Malignant cell transformation is generally accompanied by changes in their interactions with environing matrix proteins in a way to facilitate their migration and generate invasion. Our results show the binding of rat colon adenocarcinoma PROb cells to fibronectin strongly reduced when compared to normal rat intestine epithelial cells. This decrease was not due to the level of α(s)β1 integrins expressed at the surface of the cell line. However, β_1- and $\alpha_{(s)}$-associated subunits appeared to be structurally altered as shown by immunoprecipitation followed by electrophoresis. Pulse chase experiments using ^{35}S methionine evidenced differences in the biosynthesis of β1- and α (s) associated integrins: normal epithelial IEC18 cells required 16 h for maximal biosynthesis of the completely mature β1 subunit, while PROb cells did it within 4–6 h. Studies using endoglycosidases O, H, D, and N glycanase confirmed that the molecular weight alterations were due to abnormal glycosylation and suggested that α(s)β1 integrins of PROb cells could bear both mature complex and immature high mannose types while IEC18 cells borne only mature complex type oligosaccharidic chains. Treatment of both cell types with castanospermine, an inhibitor of N-glycosylation, reduced the differences observed in their adhesion to the fibronectin without significantly affecting β1 receptors expression at the cell surface. These results strongly suggest a role of the glycosylation of β1 receptors in the adhesion of rat colon adenocarcinoma PROb cells to fibronectin substrata.

Key words: fibronectin receptors, β_1 integrin glycosylation, rat colon carcinoma, matrix proteins

INTRODUCTION

Many functions of multicellular organisms involve complex interactions of cells with extracellular matrices (ECM), including growth, morphology, differentiation, wound healing, and tumor metastasis [Thiery et al., 1985; Nicolson, 1987; Lévesque et al., 1991; Kubota et al., 1992; Ruoslahti, 1992; Matsushima et al., 1992]. These interactions occur through specific cell surface receptors, many of which belong to a large superfamily of related molecules called integrins. Integrins are noncovalent, heterodimeric complexes consisting of α and β subunits. To date, 15 α and 8 β subunits have been described, giving rise to at least 21 distinct integrin receptors [Humphries, 1990; Ruoslahti et al., 1994; Hynes, 1992; Palmer et al., 1993]. β_1 integrins appear to form the major class of ECM receptors among the integrin superfamily, including those for fibronectin [Pytela et al., 1985], vitronectin [Neugebauer et al., 1992], laminin [Ignatius and Reichardt, 1988], and collagen I and IV [Tomaselli et al., 1987; Kramer et al., 1989].

Presently, there is some evidence for a role of glycosylation in cell interactions with ECM. A decrease in cell-surface sialic acid can result in increased or decreased cell attachment to basement membrane proteins (collagen IV and fibronectin) [Dennis et al., 1982; Morgenthaler et al., 1990; Kemmner et al., 1992]. Studies have also shown that glycosylation may directly influence integrin binding functions. Recently, α-galactosyl residues were identified as the laminin-binding determinant on an integrin α subunit, whereas β chain glycans were associated with cell spreading rather than with cell adhesion [Chammas et al., 1993]. Moreover, a decrease in

Abbreviations used: ECM, extracellular matrix; Endo D, endo-β-N-acetylglucosaminidase D; N glycanase, peptide-N-glycosidase F; Endo H, endo-β-N-acetylglucosaminidase H; PBS, phosphate-buffered saline; PMSF, phenylmethylsulfonyl fluoride; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; BSA, bovine serum albumin.

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the sialylation stage of β_1 integrin in a B16 melanoma cell variant [Öz et al., 1989], and of integrin $\alpha_5\beta_1$ in phorbol ester-treated K562 erythroleukemia cells, reduced their adhesion properties [Symington et al., 1989]. However, these interactions are not well defined, and some results are rather controversial. Akiyama et al. [1989] used glycosylation processing inhibitors to demonstrate that complete oligosaccharide processing of the fibronectin receptor is important for its binding function but not for receptor assembly or insertion into the plasma membrane. Conversely, Koyama and Hughes [1992] have demonstrated that terminal oligosaccharide processing of $\alpha_5\beta_1$ integrin subunits is not required for fibronectin binding.

PROb cells have been isolated from a single colonic adenocarcinoma chemically induced in BDIX rats, and gives progressive tumors which metastasize after s.c. injection into syngeneic animals [Caignard et al., 1985]. In a previous study, we showed that PROb cells weakly adhered to a fibronectin-coated substrate via RGDS-dependent integrins [Harb et al., 1992]. We demonstrate here that this adhesion, mainly mediated by β_1 integrins, is lower than that observed with normal rat epithelial cells from the digestive tract. This property could be due to the altered glycosylation of $\alpha(s)\beta1$ integrins beared by PROb cells compared to that of normal rat epithelial cells, since treatment of both cell types with castanospermine, an inhibitor of N-linked glycosylation, reduced this difference in cell adhesion to fibronectin without affecting the receptors expression.

MATERIALS AND METHODS

Materials and Antibodies

Castanospermine, Endoglycosidase H (of *Streptomyces plicatus* from *Streptomyces lividans*), N glycosidase F (from *Flavobacterium meningosepticum*), endoglycosidase D (from *Diplococcus pneumoniae*), and O glycosidase (from *Diplococcus pneumoniae*), were obtained from Boehringer Mannheim (Meylan, France). [^{35}S]methionine-cysteine (1217 Ci/mmol), [^{3}H]thymidine (2 Ci/mmol) and Na^{125}I were purchased from Du Pont-New England Nuclear (Boston, MA). Anti-rabbit IgG coupled to agarose was obtained from Sigma Chemical Company (St. Louis, MO).

Rabbit polyclonal antisera raised against rat α_5 and β_1 integrin subunits were kindly provided by Dr Johansson (Upssala University, Sweden) [Johansson et al., 1987]. Rabbit polyclonal antisera specific for C terminus of chicken integrin α_3 [Hynes et al., 1989] and β_1 [Marcantonio and Hynes, 1988] subunits were kind gifts from Dr. Hynes (Massachussets Institute of Technology, Cambridge, MA). Rabbit polyclonal antibodies against the α_v integrin subunit extracellular domain were kindly provided by Dr. Reichardt (University of California, San Francisco, CA) [Bossy and Reichardt, 1990].

Cell Culture and Metabolic Labeling

PROb cells (a gift from Dr. F. Martin, Dijon, France) were grown in monolayer cultures at 37°C in RPMI 1640 medium (Gibco, Grand Island, NY) supplemented with 5% fetal calf serum and 2 mM glutamine. IEC6 (normal rat small intestine epithelial cells) and IEC18 (normal rat ileum epithelial cells) were obtained from ECACC (Salisbury, UK). They were grown in DMEM supplemented with 10% fetal calf serum, 0.1 IU/ml insulin and 2 mM glutamine. Cells were passaged with 0.025% trypsin and 0.02% EDTA and routinely tested for mycoplasma contamination with Hoechst labeling [Chen, 1977].

In pulse-chase experiments, PROb and IEC18 cells were first cultured 30 min in methionine-cysteine-deficient RPMI 1640 medium containing 5% dialyzed fetal calf serum and 2 mM glutamine. Cells were labeled for 20 min with 100 μCi/ml of [^{35}S]methionine-cysteine in the same medium. The labeled medium was then removed and cells were washed three times and incubated with RPMI 1640 complete medium with unlabeled methionine-cysteine for several chase periods. Labeled cells were washed with phosphate-buffered saline (PBS), scraped off, pelleted, and treated for immunoprecipitation.

Surface Iodination

Monolayer cultures were washed with PBS, scraped off, and pelleted. Cells were suspended in 0.5 ml of PBS and surface-iodinated in glass vials coated with 100 μg of iodogen [Nakamura et al., 1988] by adding 0.5 mCi of Na^{125}I. After 15-min incubation at room temperature, cells were pelleted and washed in PBS.

Immunoprecipitation

Immunoprecipitation was performed as described by Akiyama and Yamada [1987]. Briefly, labeled cells were detergent extracted on ice

with 0.4 ml of ice-cold 2% Triton X-100 in PBS supplemented with 2 mM phenylmethylsulfonyl fluoride (PMSF), 2 μg/ml pepstatin, 2 mM 1,10 phenanthroline, and 10 μM E-64 for 30 min. The extract was centrifuged for 15 min at 13,000*g*. The supernatant solution was then precleared with 50 μl of anti-rabbit IgG agarose. The agarose beads were pelleted by centrifugation and discarded to eliminate nonspecifically adsorbing materials. The supernatant was then incubated with rabbit polyclonal antibodies against integrin subunits (1/100). After 4-h incubation, with constant end-over-end mixing, immune complexes were immunoprecipitated by adding 50 μl of anti-rabbit IgG agarose preadsorbed with unlabeled Triton X-100 cell extract. The agarose-bound immune complexes were washed with 1% Triton X-100 in PBS containing protease inhibitors and extracted from the agarose beads by boiling in nonreducing sample buffer for electrophoresis.

Glycosidase Digestions

After immunoprecipitation, the agarose-bound immune complexes were boiled 10 min in the appropriate buffer and treated with 5 milliunits of endoglycosidase H in 50 μl of 50 mM sodium acetate, 0.01% SDS, pH 5.7, or 2 units of N glycosidase F in 50 μl of 125 mM sodium phosphate, 0.1% SDS, 0.5% Nonidet P40, pH 8.5, or 5 milliunits of endoglycosidase D in 50 μl of 50 mM sodium citrate, 0.01% SDS, pH 5.3, or 1 milliunit of O glycosidase in 25 μl of SDS 0.1% + 25 μl of 25 mM Tris maleate, 1% Nonidet P40 + 6 μl of 250 mM Tris, pH 6 at 37°C for up to 24 h. The supernatant was discarded and the agarose-bound immune complexes were detached by boiling in nonreduced sample buffer for electrophoresis.

SDS Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Samples were heated at 100°C for 5 min in Laemmli buffer without reducing agent, applied to 5–10% or 7% polyacrylamide gel and subjected to electrophoresis [Laemmli, 1970]. Radioactive bands were located by autoradiography using Hyperfilm-MP (Amersham, Bucks, UK), or phosphorimager 445SI (Molecular Dynamics, Sunnyvale, CA).

Cell Attachment Assays

Plates were coated with bovine fibronectin in PBS overnight at 4°C and then with 1% BSA for 1 h at room temperature. BSA-coated wells were used as controls. Cells were grown in complete medium supplemented with 10 μCi/ml [^{3}H]thymidine for the last 18 h. Cells were trypsinized, washed 3 times in binding buffer (20 mM Tris-HCl, pH 7.4, 135 mM NaCl, 5 mM KCl, 1.8 mM D-glucose, 2 mM glutamine, 1% BSA, 1 mM $MnCl_2$) [Elices et al., 1991], and resuspended in the same buffer at 5×10^5 cells/ml. One hundred ml of suspension were added to the wells coated with different substrates and allowed to attach for 90 min at 37°C. Unbound cells were removed by gentle washing with PBS. Bound cells were dissolved in 0.1 N NaOH and quantitated by liquid scintillation spectrometry in a Beckman LS2800β counter. Triplicate assay points were averaged, and nonspecific BSA binding value was subtracted.

Flow Cytometry Analysis

Integrin expression on cells was determined by indirect immunofluorescence using a FACScan (Becton Dickinson, San Jose, CA). Briefly, 2×10^5 cells were first incubated with the appropriate primary antibody for 45 min at 4°C (or the same volume with PBS-gelatin 0.1% for the control), then washed twice with PBS-gelatin 0.1% and incubated with an affinity purified fluorescein isothiocyanate labeled goat anti-rabbit F(ab')2 fragment (Sigma, La Verpillère, France) diluted to 1:200 for 45 min at 4°C.

Treatment of Cells With Castanospermine

Subconfluent cells were grown at 37°C for 36 h in the presence of 200 μg/ml of castanospermine. In these conditions, no cell death was observed by trypan blue exclusion. Cells were labeled with ^{3}H thymidine before cell adhesion assays as described above.

RESULTS

PROb Tumor Cells Less Adhered to Fibronectin Than Normal Rat Epithelial Cells

PROb cells have been previously shown to slightly attach to fibronectin (30% at 10 μg/ml of fibronectin) by interacting with the RGDS-containing cell-binding domain [Harb et al., 1992]. Figure 1 shows that in the same conditions, normal rat epithelial cells IEC6 and IEC18 strongly adhered to fibronectin in comparison to

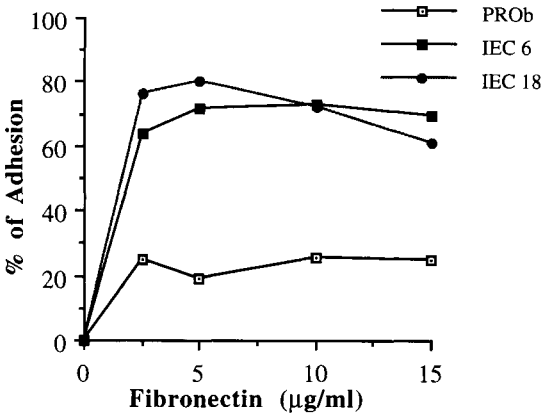

Fig. 1. Adhesion of PROb, IEC6, and IEC18 cells to fibronectin. Cells were labeled for 18 h with complete medium containing 10 μCi/ml [^{3}H] thymidine. 5 × 10^4 cells/well were then added to plates, precoated with different concentrations of fibronectin, and allowed to adhere for 90 min as described under Materials and Methods. Results are the mean of two experiments.

PROb cells. A maximal adhesion was observed at 2.5 μg/ml of fibronectin when adhesion was approximately 2.5 and 3 fold higher for IEC6 and IEC18 respectively than for tumor PROb cells.

Cell Surface Expression of α(s) and β_1 Subunits

Because the interactions between cells and fibronectin are mostly mediated by the β1 integrins family, we investigated the expression of these integrins on the cell surface of PROb and normal rat epithelial cells. β1 integrins were equally expressed on the cell surface of tumor and normal rat IEC18 cells, as shown by cytofluorimetry (Fig. 2). Similar results were obtained with IEC6 cells (not shown). The immunoprecipitation with an antiserum against the β_1 subunit showed under nondenaturing conditions, that the β_1 subunit in PROb cells had an Mr of 105,000 and the associated α(s) subunit(s) an Mr of about 125,000 (Fig. 3, lane 1). On the opposite, α(s)β_1 integrins of IEC18 and IEC6 showed a slower electrophoretic mobility when compared to that of PROb cells (Fig. 3, lanes 2 and 3) which corresponded to an Mr of 116,000–120,000 for β1 and an Mr of 150,000 for α(s) in agreement with previously published data [Johansson et al., 1987]. The use of antisera against α_3 (Fig. 3, lanes 4 and 7), α_v (Fig. 3, lanes 5 and 8), and α_5 (Fig. 3, lanes 6 and 9) subunits showed the same abnormal electrophoretic mobility of these subunits in PROb when compared with IEC18 cells.

Biosynthesis of β_1 Integrins in PROb and IEC18 Cells

As this generally faster electrophoretic mobility could reflect a defect in α(s)β_1 integrins biosynthesis, a pulse-chase labeling protocol was used to investigate the processing of β_1 polypeptides and associated α subunits. In a first set of experiments, we performed short chase periods. In PROb cells, immediately after the pulse, anti-β_1 specific antibody precipitated a preβ_1 subunit which was very weakly associated with α(s) subunit(s). The association became maximal after at least 2 h of chase. However, no change in the apparent molecular mass of β_1 and α(s) could be detected, even after 24 h of chase (Fig. 4). On the opposite, in IEC18 cells, the maturation of preβ1 was slower since β1 subunit was only observed at point 24 h chase time (results not shown). Thus, in order to more specify the biosynthesis mechanism of β1 subunit in IEC18 cells, we performed another set of experiments and realized several chase periods between 2 and 16 h. The results obtained confirmed the fast association of the β1 subunit with α(s) ones in PROb cells and the absence of change in the apparent molecular mass of α(s)β1 integrins (Fig. 5B). On the opposite, β1 subunit underwent to mature in IEC18 cells within 4 to 8 h and after 16 h of chase, only nearly 50% of β1 was matured (Fig. 5A). Finally, we performed ultimate experiments with IEC18 cells increasing the pulse time (2 h) and extending chase period until 40 h. Figure 6 shows that at this time, β1 subunit was not completely matured. Moreover, in IEC cells, α(s) subunits were matured within 2 h, earlier than β1 subunit, (Fig. 5A), as previously described [Akiyama et al., 1990].

Analysis of Cell Surface Integrin β_1 Receptors Glycosylation on PROb Cells and IEC18

To confirm that the differences in the electrophoretic mobility observed between cancerous and normal cells were due to the glycosylation state of these integrins, we treated the extracts immunoprecipitated with the anti-β1 antibody by several endoglycosidases. O and endo D glycosidases did not modify the electrophoretic profile of the subunits from both cell lines (data not shown). However, after N glycanase treatment, the α and β subunits collapsed to the molecular weights corresponding to carbohydrate-free poypeptides (Fig. 7A and B, lane 3), indicating that

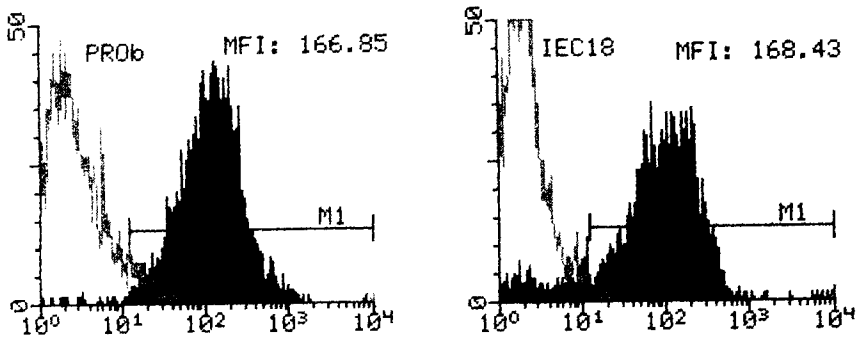

Fig. 2. β_1 integrins expression in PROb and IEC18 cells. 2×10^5 PROb **(left)** or IEC **(right)** cells were preincubated with (black histograms) or without (empty histograms) anti-β1 antibody as described under Materials and Methods. After washing, cells were incubated with the second FITC-labeled antibody and analysed with a FACScan cytofluorimeter. MFI = mean fluorescence intensity.

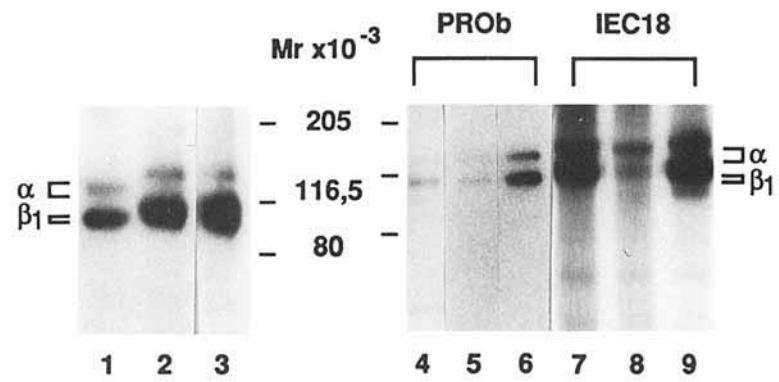

Fig. 3. Surface labeling of PROb, IEC18, and IEC6 cell integrins. Radioiodinated PROb (*lanes 1, 4–6*), IEC18 (*lanes 2, 7–9*), and IEC6 (*lane 3*) were extracted with 2% Triton X-100 in PBS and immunoprecipitated with anti-integrin β_1 (lanes 1–3), α_3 (lanes 4 and 7), α_v (lanes 5 and 8), and α_5 (*lanes 6 and 9*) sera. Immune precipitates were subjected to 5–10% or 7% SDS-PAGE under nonreducing conditions, followed by autoradiography.

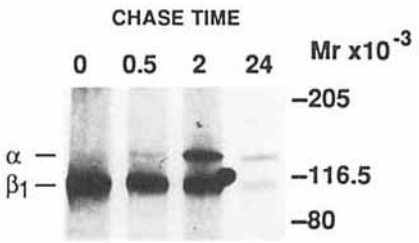

Fig. 4. Pulse-chase analysis of α(s)β_1 integrin subunit processing in PROb cells. PROb cells were metabolically radiolabeled for 20 min with [^{35}S]methionine and then incubated in nonradioactive medium for up to 24 h. Cells were extracted at the times indicated (time "0" was the beginning of the chase period). Extracts were immunoprecipitated with anti-β_1 serum and analyzed by 5–10% SDS-PAGE under nonreducing conditions, followed by autoradiography.

these subunits bore only N glycans and no O glycans on the two cell types. Moreover, PROb α(s)β1 integrins were only partially deglycosylated by endo H treatment (Fig. 7A, lane 2). In contrast, β1 containing integrins from IEC18 cells were totally insensitive to endo H treatment (Fig. 7B, lane 2), suggesting that the glycosylation of PROb integrins is different and apparently less mature than that of normal rat ileum epithelial cells.

Effect of Castanospermine on Cell Adhesion to Fibronectin

We finally attempted to demonstrate that the functionality of β1 integrins in PROb cells was associated to their glycosylation state and studied the effect of a precocious glycosylation inhibitor, the castanospermine, on the expression, and functionality of these receptors. Figure 8 shows that the treatment of both cell lines with 200 μg/ml of castanospermine for 36 h slightly reduced the expression of β1 integrin subunit on PROb and IEC18 cells as shown by cytofluorimetry. However, this treatment induced a noticeable decrease in the IEC18 cell adhesion to fibronectin and conducted to an adhesion level nearly

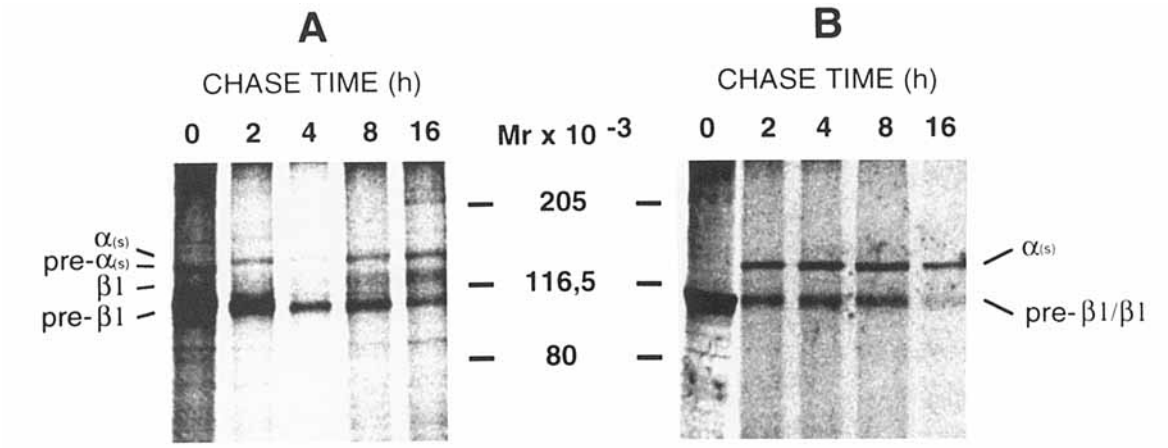

Fig. 5. Pulse-chase analysis of $\alpha(s)\beta_1$ integrin subunit processing in IEC18 and PROb cells. IEC18 (**A**) and PROb (**B**) cells were metabolically radiolabeled for 20 min with [^{35}S]methionine and then incubated in nonradioactive medium for up to 16 h. Cells were extracted at the times indicated (time "0" was the beginning of the chase period). Extracts were immunoprecipitated with anti-β_1 serum and analyzed by 7% SDS-PAGE under nonreducing conditions. Labelled proteins were detected by Phosphorimager 445SI.

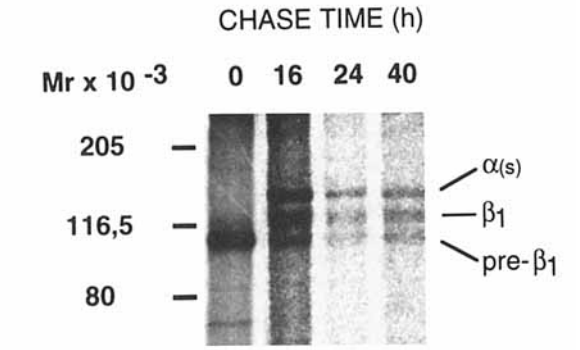

Fig. 6. Pulse-chase analysis of $\alpha(s)\beta_1$ integrin subunit processing in IEC18 cells. IEC18 cells were metabolically radiolabeled for 120 min with [^{35}S]methionine and then incubated in nonradioactive medium for up to 40 h. Cells were extracted at the times indicated (time "0" was the beginning of the chase period). Extracts were immunoprecipitated with anti-β_1 serum and analyzed by 7% SDS-PAGE under nonreducing conditions. Labelled proteins were detected by Phosphorimager 445SI.

similar to that of PROb cells before castanospermine treatment. This observation was more pronounced at lower fibronectin concentrations (2.5 and 5 μg/ml) (Fig. 9).

DISCUSSION

Transformation of normal cells to malignant cells results in some changes which are thought to be related to the increased propensity of these cells to migrate and invade. The expression and distribution of plasma membrane fibronectin receptors largely influence these cell functions. The inhibition of the receptors by exogenous RGD peptides abolishes substrate binding and migration of embryonal, epithelial, and tumor

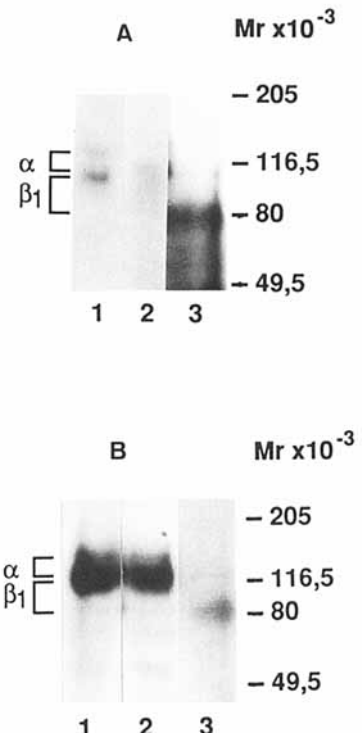

Fig. 7. Enzymatic sensitivity of $\alpha(s)\beta_1$ integrin subunits. Detergent-soluble extracts of surface iodinated PROb (**A**) and IEC18 (**B**) cells were immunoprecipitated with anti-β_1 antibody. The immune complexes were incubated overnight at 37°C with endoglycosidase H (5 mU) (*lanes 2, A and B*), N glycosidase F (2 U) (*lanes 3, A and B*), or let without treatment as controls (*lanes 1, A and B*) and analyzed by 5–10% SDS-PAGE under nonreducing conditions, followed by autoradiography.

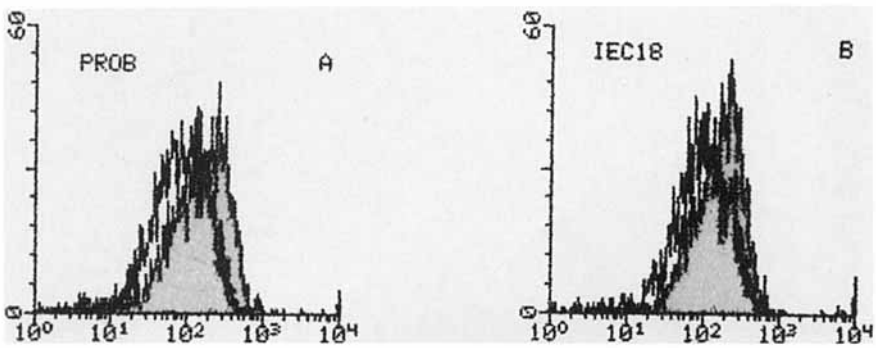

Fig. 8. Effect of castanospermine on β1 subunit expression in PROb and IEC18 cells. PROb (A) and IEC18 (B) cells were treated with 200 μg/ml of castanospermine for 36 h, then preincubated with anti-β1 antibody as described under Materials and Methods. After washing, cells were incubated with the second FITC-labeled antibody and analysed with a FACScan cytofluorimeter. Control cells: (gray histograms); castanospermine treated cells: (empty histograms).

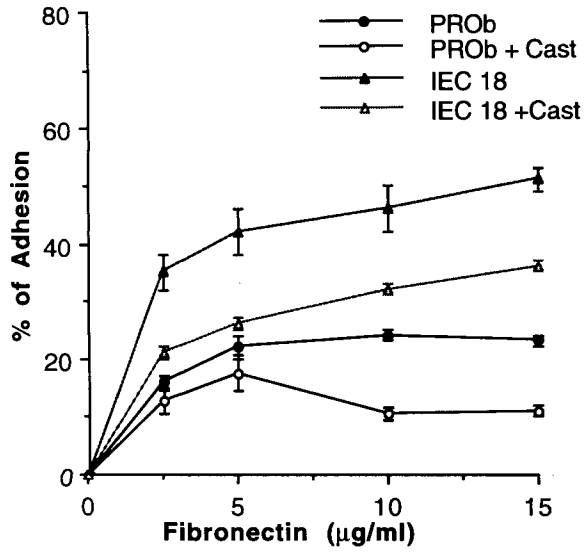

Fig. 9. Effect of castanospermine on cell adhesion to fibronectin PROb and IEC18 cells were treated with 200 μg/ml of castanospermine for 36 h, then labeled with ^3H thymidine, further added to fibronectin precoated plates and allowed to adhere for 90 min as described under Materials and Methods. Results are the mean of two experiments.

cells [Humphries et al., 1986; Savagner et al., 1986; Gehlsen et al., 1988].

In a previous work, we showed the existence of an inverse correlation between the in vivo tumorigenicity of several clones derived from the same parental rat colon cancer cell line and their adhesion to fibronectin. Nevertheless, the interaction of these cells with fibronectin was rather weak [Harb et al., 1992]. As altered glycosylation frequently accompanied cell transformation [Nicolson, 1984; Dennis et al., 1987], we studied in this paper, the glycosylation state of α(s)β1 integrins of PROb cells in comparison of normal rat epithelial cells and its role on the function of these receptors.

Our results show that PROb cells adhered less to fibronectin than both normal epithelial cell lines used and confirm the observations made on the role of fibronectin receptors and especially the α5β1 integrin in the tumorigenic phenotype. Thus, Plantefaber and Hynes [1989] showed a decrease in cell adhesion to fibronectin concomittantly to a decrease of α5β1 integrin during viral transformation. Similarly, the transfection of this integrin in CHO cells [Giancotti and Ruoslahti, 1990] and in HT29 human tumor colonic cells [Varner et al., 1992; Stallmach et al., 1994] decreases tumorigenicity of these cells. Although our results show that the β1 integrins are equally distributed on both normal and cancerous cell types, we can't exclude that differences could exist in the expression of other fibronectin receptors belonging to β3 or β6 integrin families. Specific antibodies against rat subunits are necessary to confirm this point.

We found that the kinetics and maturation of β1 subunit glycosylation was altered when compared to that of normal IEC18 cells. Pre-β1 polypeptides processing was essentially completed within less than 4 h in PROb cells while, in IEC18 cells, radiolabeled mature β1 polypeptides did not begin to appear until 4 h after labeling, were maximally present for 12–16 h after labeling (Fig. 5B) and their maturation was not completely achieved after 40 h of chase (Fig. 6). This difference in the biosynthesis of β1 integrin receptors between normal and transformed cells was similar to that observed in human normal and transformed fibroblasts [Akiyama et al., 1990]. This process could relate to transformation or reflect the inhibition of mature forms total processing. In fact, we detected a 105 kDa form of the β_1 subunit expressed on the cancerous PROb cell membrane but not on

normal rat ileum and small intestine epithelial cells ones. Von Lampe et al. [1993] have also demonstrated that malignant transformed epithelial cells of colorectal carcinoma express a 105 kDa precursor form of the β_1 chain not found in their normal conterparts. Our results could reflect a general process due to malignant transformation in colon carcinoma.

The difference in the electrophoretic mobility of the PROb cell $\alpha(s)\beta_1$ subunits was really related to glycosylation since N-glycanase digestion resulted in molecular weights which have already been reported for α and β polypeptides [Argraves et al., 1987]. Moreover, after N-glycanase digestion, molecular weights of α and β polypeptides of PROb cells and normal epithelium of rat ileum (IEC18 cells) were identical. It might seem that in PROb cells, the processing stopped early in maturation since the molecular weights of the precursors and final forms were similar to those of the $\alpha_5\beta_1$ integrin of a ricin-resistant variant of baby hamster kidney cells (Ric14) which expresses immature $Man_5GlcNAc_2$ glycans [Koyama and Hughes, 1992]. However, $\alpha(s)\beta_1$ glycans from PROb cells were different, being insensitive to endoglycosidase D [Mizuochi et al., 1984] and only partially sensitive to endo H.

Endo H cleaves high mannose and hybrid oligosaccharides [Maley et al., 1989], which would suggest that $\alpha(s)\beta_1$ glycans were processed to partially complex forms and bore at least two types of glycans, one endo H-sensitive and the other endo H-insensitive. This could be illustrated by the production of glycans based on GlcNAcβ1 $\rightarrow$ $2Man_5GlcNAc_2$ (endo H-sensitive) and GlcNAcβ1 $\rightarrow$ $2Man_3GlcNAc_2$ (endo H-insensitive) structures [Maley et al., 1989]. A majority of GlcNAcβ1 $\rightarrow$ $2Man_3GlcNAc_2$ should probably be present since endo H digestion caused only a slight decrease in the molecular weight of $\alpha(s)\beta_1$ integrins (Fig. 7A). Our hypothesis was reinforced by the study of the PROb glycoprotein pE4 cloned in our laboratory [Chadéneau et al., 1994]. This glycoprotein exhibits heterogeneity in its glycan portion when compared to MAD B mammary carcinoma cells. Monosaccharide analysis showed that pE4 from PROb cells contains Man, GlcNAc, and Fuc in a molar ratio of 3:3:0.75 and no galactose, whereas pE4 from MAD B cells contains Man, GlcNAc, Gal, and Fuc in a molar ratio 3:6:3.5:2 [Chadéneau et al., 1995]. The glycosylation defect could be due to deficiency in mannosidase II and/or GlcNAc transferase II.

Nonetheless, the glycosylation type of $\alpha(s)\beta1$ integrins beared by PROb cells could partially explain their low adhesive properties to fibronectin. In fact, glycosylation inhibition by castanospermine slightly altered the expression of $\beta1$ integrin subunit suggesting that the alteration of the glycosylation did not interfere with biosynthesis, assemblage, and surface expression of these receptors, in good agreement with reports from laboratories with various cell lines [Akiyama et al., 1989; Koyama and Hughes, 1992; Hotchin and Watt, 1992; Decastel et al., 1993]. At the opposite, the alteration in glycosylation strongly inhibited the adhesion of normal IEC18 cells to fibronectin confirming the necessity, at least in our model, of mature glycosylation for maximal functionality of $\beta1$ receptors.

Integrin receptors can transduce biochemical signals from the extracellular matrix to the cell interior in order to modulate cell behavior [Hynes, 1992; Juliano and Haskill, 1993]. Thus, endogenous activation of protein kinase C in response to cell adhesion to fibronectin precedes cell spreading and migration [Vuori and Ruoslahti, 1993]. Furthermore, fibronectin can stimulate the proliferation of quiescent melanoma cells and integrins as $\alpha_5\beta_1$ are involved in this response [Mortarini et al., 1992]. Alterations of integrin glycosylation have often been demonstrated in transformed cells and could interfere with signal transduction, thus accounting for some aspects of the abnormal behavior of these cells.

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