

Differential expression of glycoproteins containing α -D-galactosyl groups on normal human breast epithelial cells and MCF-7 human breast carcinoma cells

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Summary

Cell surface glycoproteins were isolated from the lysates of ¹²⁵I-labeled normal human mammary epithelial cells (NHMEC) and from the human breast carcinoma cell line MCF-7, of blood-group O phenotype, by affinity chromatography on *Griffonia simplicifolia* I lectin-Sepharose. Specific elution of glycoproteins from the column with methyl α -D-galactoside suggests the presence of α -D-galactosyl groups on these moieties. SDS-PAGE analysis of isolated glycoproteins revealed both quantitative and qualitative differences between glycoproteins from normal and malignant cells. Three major glycoproteins of *M_r* 180 kDa, 85 kDa and the 44 kDa were obtained from

MCF-7 cells. The 180-kDa glycoprotein was absent in NHMEC and the 44-kDa glycoprotein was very weakly expressed in these cells. The only glycoprotein which was found in almost equal amount in the lysate from both normal and malignant cells was the 85-kDa glycoprotein. These results indicate differences between normal human mammary epithelial cells and one kind of malignant human mammary epithelial cells, in the expression of glycoproteins containing α -D-galactosyl groups, irrespective of blood-group phenotype; they also demonstrate that α -D-galactosyl groups are expressed in a very restrictive manner on the surface of this tumor cell line.

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Abbreviations: BSA, bovine serum albumin; FITC-fluorescein isothiocyanate; D-Gal, D-galactose; GS I, *Griffonia simplicifolia* I lectin; GS I-B₄, *Griffonia simplicifolia* I-B₄ isolectin; Me α DGal, methyl α -D-galactoside; NHMEC, normal human mammary epithelial cells; PAGE, polyacrylamide gel electrophoresis; PBS, 0.01 M phosphate buffer (pH 7.1) with 0.15 M NaCl and 0.02% azide (this buffer was free of divalent cations). PBS/Ca²⁺, PBS containing 0.1 mM CaCl₂; PMSF, phenylmethane sulfonyl fluoride; SDS, sodium dodecyl sulfate.

Keywords: α -D-galactose; breast; cancer

Introduction

The expression of specific oligosaccharide structures on tumor cells may contribute to their tumorigenicity or metastatic behavior [1]. Varani et al. [2] have shown that the expression of α -D-galactosyl residues on the cell surface is strongly correlated with metastatic potential of murine fibrosarcoma cells. Highly

metastatic cells from a number of different tumors expressed high levels of α -D-galactosyl groups whereas low malignant cells from the same tumors did not. Moreover, results with the same cells indicated that these α -D-galactosyl groups were located on glycoproteins which may play a role in tumor cell attachment to collagen [3]. Castronovo et al. [4] reported the presence of α -D-galactosyl groups on another highly malignant murine cell line, MO4. Their results also suggested that these groups may play a role in the formation of metastases, inasmuch as they were able to reduce lung colonization using anti- α -D-galactosyl antibodies. While previous studies have documented a strong, positive relationship between cell surface expression of α -D-galactosyl groups and malignant potential among murine tumor cell lines, much less work has been done in humans. Recently Castronovo et al. [5] also demonstrated the presence of α -D-galactosyl groups on certain human tumor cell lines derived from human brain, breast and chorion as well as on some human breast cancer cells obtained at biopsy. These carbohydrate groups on choriocarcinoma cells (BEWO) were implicated in the process of attachment of these cells to laminin and to the vascular wall. While these previous studies support the notion that, in human as in mouse, the expression of α -D-galactosyl groups is associated with the malignant phenotype, additional studies are necessary to determine the significance of such a relationship.

An important question to be addressed concerns the nature of the tumor cell surface molecules which bear the α -D-galactosyl groups. Terminal α -D-galactosyl groups are expressed on murine laminin from EHS sarcoma [6] and studies by McCoy et al. [7] indicated that laminin was responsible for at least some of the residues on murine fibrosarcoma cells. However, laminin probably accounts for only a minor portion of the α -D-galactosyl groups on murine cells. Grimstad and Bosnes [8] indicated that α -D-galactosyl groups which contribute to tumor cell attachment are located

on molecules other than laminin. However, these molecules have not been identified. Further, laminin from human cells does not contain terminal α -D-galactosyl groups [9].

In order to address these questions, we have developed a procedure for the isolation of glycoproteins bearing α -D-galactosyl groups from the human cells. Our previous studies with glycoproteins containing α -D-galactosyl groups on the surface of macrophages showed that the expression of these carbohydrates is limited to two major glycoproteins (Petryniak et al., unpublished data). Utilizing this procedure, we have compared the expression of glycoproteins bearing α -D-galactosyl groups, on normal human breast epithelial cells and malignant MCF-7 human breast carcinoma cells. We found that there are few glycoproteins with α -D-galactosyl groups on the surface of each kind of cell. Furthermore, there are substantial differences in the electrophoretic mobility profiles of the glycoproteins isolated from the normal and malignant cells.

Materials and Methods

Cells

Normal human mammary epithelial cells (NHMEC) and breast carcinoma cells. NHMEC were obtained from reduction mammaplasty of an individual with blood-group B phenotype and established in monolayer culture as described by Soule and McGrath [10]. Briefly, fresh tissue was dissected into small pieces with the aid of a scalpel and then dissociated in collagenase and hyaluronidase. Fibroblasts were removed by differential adhesion. The dissociated cells were then plated on plastic using a mixture of Dulbecco's modified minimal essential medium and Ham's F12 medium. The medium was prepared de novo without inclusion of CaCl_2 in the balanced salt solution. The medium was supplemented with 100 units/ml penicillin, 100 $\mu\text{g/ml}$ streptomycin, cholera enterotoxin (100 ng/ml), epidermal growth factor (20 ng/ml), horse serum (5%), insulin (10 $\mu\text{g/ml}$) and cortisol (1.4×10^{-6} M). Finally, CaCl_2 was added to

bring the final concentration to 2 mM for plating and cells were maintained in identical media with 16 μM CaCl_2 after attachment.

MCF-7 human breast carcinoma cells were obtained from the American Type Culture Collection. This cell culture was established from an individual of blood group O phenotype. They were maintained in monolayer culture using minimal essential medium of Eagle with Earle's salts, non-essential amino acids, 100 units/ml of penicillin, 100 $\mu\text{g}/\text{ml}$ of streptomycin and 10% fetal bovine serum as culture medium. The cells were grown at 37°C and 5% CO_2 and subcultured by trypsinization as required.

Reagents

^{14}C -Labeled molecular weight marker-proteins were obtained from BRL Inc. (Bethesda, MD). These included: myosin, 200 kDa; phosphorylase B, 97.4 kDa; bovine serum albumin, 68 kDa and ovalbumin, 43 kDa. Iodogen was from Pierce Rockford, IL. *Griffonia simplicifolia* I lectin was purified from seeds provided by Calbiochem as previously described [11]. The lectin was coupled to Sepharose 4B by the CNBr procedure [12]. The conjugate contained approximately 1.1 mg of lectin per ml of settled beads.

Cell surface labeling

Both NHMEC and MCF-7 cells were surface-labeled with ^{125}I iodine using the iodogen technique [13]. Briefly: to approximately 5×10^6 cells suspended in 2 ml of PBS in a glass vial coated with 800 μg of iodogen, 1.5 mCi of ^{125}NaI (carrier free) was added. The suspension was mixed for 15 min at room temperature and cells were transferred to polypropylene tubes and washed four times with 15 ml of PBS, sedimenting for 5 min at $400 \times g$ each time.

Isolation of glycoproteins

All purification steps were carried out at 4°C, unless stated otherwise. ^{125}I -Labeled cells were lysed and subjected to affinity chromatography. Lysis was carried out on an ice

water-bath for 3 min (pH 8.2) in 0.01 M Tris-HCl buffer containing 0.15 M NaCl, 1% BSA and 4% Triton X-100. Freshly prepared protease inhibitors, were added to this lysing buffer just prior to lysis. Inhibitors included aprotinin (10 μg), antipain (50 μg), benzamidine HCl (100 μg), chymostatin (100 μg), iodoacetamide (1 mM) leupeptin (10 μg), pepstatin (100 μg), 1,10-phenanthroline (5 mM) and PMSF (2 mM). (Numbers in parentheses represent final concentrations of inhibitor per ml of buffer). The lysate was centrifuged for 15 min in a microfuge at $1500 \times g$. The supernatant from the lysate was applied immediately to the *Griffonia simplicifolia* I lectin-Sepharose column (0.7×8 cm). The column was equilibrated with PBS/ Ca^{2+} and washed successively with buffers (see Fig. 1) until the cpm dropped below 1000 per fraction. Absorbed material was specifically eluted with methyl α -D-galactoside and analyzed by SDS-PAGE.

Acrylamide gel electrophoresis was carried out according to Laemmli [14] in 1.5 mm thick 7.5% acrylamide slab gels in the presence of 0.1% SDS, at constant current of 25 mA. For autoradiography, gels were dried and exposed to Kodak X-Omat XAR 5 films for 7–14 days. M_r of glycoproteins was determined from the standard curve fitted by linear regression.

Results and Discussion

In order to show that the structural difference between NHMEC and mammary carcinoma cells in the expression of the α -D-Gal epitope is irrelevant to blood-group phenotype, MCF-7 carcinoma cells from a blood group O individual were chosen. These individuals do not express the α -D-Gal epitopes inasmuch as they lack α -D-galactosyltransferase. In contrast, individuals with blood group B phenotype do contain α -D-Gal residues linked to fucosylated oligosaccharides. Therefore NHMEC were from a blood group B individual to minimize the possible differences between these two kinds of cells regarding the expression of the α -D-Gal

epitope. Affinity chromatography of lysates from ^{125}I -labeled NHMEC and from malignant MCF-7 cells on the *Griffonia simplicifolia* I lectin-Sepharose column resulted in peaks eluted specifically with methyl α -D-galactoside (Fig. 1, Panel A and B). Incorporation of ^{125}I into material eluted from the column indicates

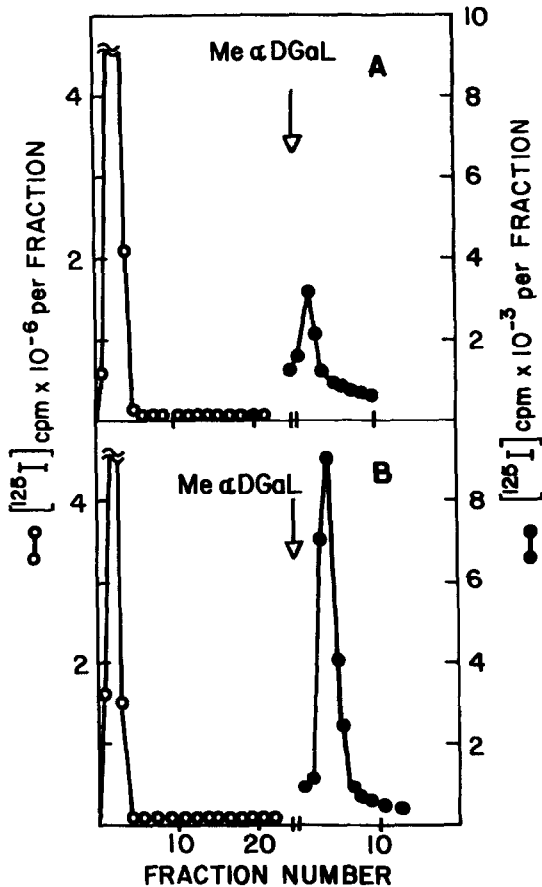


Fig. 1. Affinity chromatography on *Griffonia simplicifolia* I lectin-Sepharose of lysates from cells labeled with ^{125}I . Lysates from NHMEC (panel A) and MCF-7 cells (panel B) were applied to a column (0.7 \times 8.0 cm) equilibrated in PBS/ Ca^{2+} and was washed successively with 50 ml of PBS/ Ca^{2+} containing 1% Triton X-100 and 50 ml of PBS/ Ca^{2+} without Triton X-100. The column was equilibrated with PBS/ Ca^{2+} containing 0.2% octyl glucoside (buffer A). Fraction of 1.3 ml were collected. The flow rate was 16 ml/h. Absorbed material was eluted with 15 ml of 100 mM methyl α -D-galactoside in buffer A.

that the eluted material is probably a glycoprotein in nature and that it contains terminal α -D-galactosyl groups. There was more ^{125}I -labeled material eluted from the lysate of MCF-7 cells than NHMEC. However, an answer to the question as to whether this difference results from a different number of receptors on each kind of cell would require quantitative studies. Materials in both peaks eluted from *Griffonia simplicifolia* I lectin-Sepharose column were analyzed by SDS-PAGE (Fig. 2). Autoradiograms of the material resolved in the gel revealed both qualitative and quantitative difference between glycoproteins from normal and malignant cells. Several

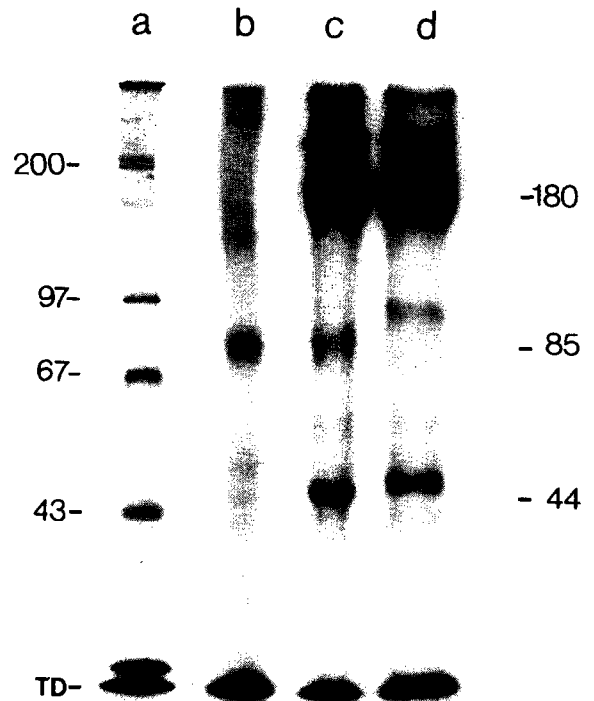


Fig. 2. Autoradiogram of SDS-PAGE of ^{125}I -labeled glycoproteins from NHMEC and MCF-7 cells. Glycoproteins were isolated by affinity chromatography on *Griffonia simplicifolia* I lectin-Sepharose and eluted with methyl α -D-galactoside. Electrophoresis was carried out in a 7.5% acrylamide gel at constant current of 25 mA. (a) molecular weight markers; (b) NHMEC; (c) MCF-7 cells (unreduced conditions); (d) MCF-7 cells (reduced with 2% DTT).

glycoproteins are expressed specifically or at higher levels in the MCF-7 cells.

Three major glycoproteins of M_r 180 kDa, 85 kDa and 44 kDa were found in the lysate from MCF-7 cells (Fig. 2c). These glycoproteins appear to be cell membrane components inasmuch as they were labeled on the surface of intact cells with ^{125}I by the insoluble iodogen reagent which can not enter the cell. Of these three glycoproteins in the tumor cell lysate, the glycoprotein of 180 kDa represents the strongest band; it was completely absent in the lysate from normal cells (Fig. 2b). This glycoprotein was also insensitive to reduction with β -mercaptoethanol (Fig. 2d) suggesting the lack of subunit structure linked by disulfide bonds. It will be of interest to determine if this glycoprotein is breast cancer-specific or whether it may be present in other types of cancer cells. The 44-kDa glycoprotein, which also gave a strong band in the lysate from MCF-7 cells, was barely detectable in the lysate from NHMEC (Fig. 2b). The 44-kDa glycoprotein from MCF-7 cells migrated as a sharp band whereas that from normal cells moved as a broader, diffuse band (Fig. 2b, c). Whether these glycoproteins from the two types of cells are the same remains to be determined? The only glycoprotein which appeared to be present in approximately equal amounts in the lysate from both kinds of cells was the 85-kDa component. Again, whether they are identical cannot be answered at this point.

These results suggest that during the process of malignant transformation of MCF-7 cells, glycoproteins containing α -D-galactosyl groups are altered and/or expressed and suggest that the α -D-Gal epitope on the MCF-7 carcinoma cells is different from the blood-group-B structure. Determination of the linkage between the α -D-Gal groups and the subterminal carbohydrate would be of obvious interest. Immunochemical studies suggest that this linkage might be α 1-3, but such an epitope has not been found in humans [15]. Its appearance in MCF-7 breast cancer cells might be of general biological significance and the glycoproteins described here might provide a tool for such

structural studies. In addition to this major component, lysates from normal cells showed additional, very weak bands of 40 kDa, 50 kDa and a diffuse zone in the range of 133-167 kDa. These were absent from lysates of the MCF-7 cells (Fig. 2c). Both the 85-kDa and 44-kDa glycoproteins from MCF-7 cells showed a decrease in their mobility after reduction with β -mercaptoethanol and their apparent M_r increased to 100 kDa and 48 kDa, respectively. This suggests that they may consist of single polypeptide chains with intramolecular disulfide bonds. The 180-kDa, 85-kDa and 44-kDa glycoproteins we isolated from MCF-7 cells are clearly different from laminin. This is in agreement with the observation of Grimstad and Bosnes who stated the α -D-galactopyranosyl end-groups occur on species of cell-surface molecules other than laminin [8].

Are the glycoproteins isolated from MCF-7 cells identical to those previously described? Carcinoembryonic antigen (CEA) is also in the range of M_r 180 kDa [16] and has been found on breast carcinoma cells [17]. However CEA as reported by Lisowska et al. [18] has a subunit structure and, moreover, does not contain α -D-galactosyl groups [19]. In only very few cases have specific glycoproteins been shown to be associated with breast carcinoma cells [20-25]. These include a 180 kDa glycoprotein defined by monoclonal antibodies as described by Ring et al. [25]; however, there are differences between the 180 kDa glycoprotein described here, and the one described previously with the same molecular weight. The apparent M_r of the glycoprotein described by Ring et al. increased to 200 kDa after reduction with β -mercaptoethanol whereas the glycoprotein we isolated was insensitive to reduction. Moreover, these investigators did not detect this glycoprotein on MCF-7 cells. A membrane glycoprotein associated with human breast cancer tissue described by Edwards et al. [21] has a M_r of 43 kDa. Whether this glycoprotein is identical to the glycoprotein of M_r 44 kDa we isolated, remains to be determined. All other glycoproteins described

previously [19,21–23] have differed in molecular weight from the glycoproteins described here.

Currently, the biological role(s) of breast cancer-associated glycoproteins remains unknown. However, involvement of molecules containing α -D-galactosyl groups in the formation of metastases has been indicated. α -D-Galactosyl groups are more strongly expressed on murine tumor cells than on those from humans [26,27]. Nevertheless, their presence on tumor cells from both species pose the question as to whether there may be an evolutionary relationship between these glycoproteins, and whether they might play a role in human tumor metastasis. Identification of molecules containing this carbohydrate group on tumor cell surfaces, as well as their full biochemical and immunochemical characterization, may provide an approach to address these questions.

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