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IDENTIFICATION OF EXPOSED SURFACE GLYCOPROTEINS OF FOUR HUMAN BLADDER CARCINOMA CELL LINES

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Three cell surface protein-specific methods were used to radiolabel the major glycoproteins of four human bladder carconoma cell lines: The well-differentiated lines RT112 and RT4 and the more anaplastic lines T24 and EJ. Five acidic glycoproteins iodinated in all lines by the lactoperoxidase/^{125}I method were designated CP-175/5.8–6.0 (apparent molecular weight $\times 10^{-3}$/p*I* of iodoprotein), GP-155/5.0–5.3, GP-145/4.9–5.2, GP-130/4.8–5.5 and GP-110/4.9–5.3. Another iodinated glycoprotein, GP-200/5.5–6.0, was prominently labelled in RT112 and RT4 but was not detected in T24 or EJ. GP-200 as well as GP-175, GP-155 and GP-145 were not detected by the galactose oxidase/NaB(^{3}H)$_4$ method and were poorly labelled by the neuraminidase-galactose oxidase/NaB(^{3}H)$_4$ and NaIO$_4$/NaB(^{3}H)$_4$ labelling methods. The major sialogalactoproteins identified in the four lines by the neuraminidase-galactose oxidase/NaB(^{3}H)$_4$ and NaIO$_4$/NaB(^{3}H)$_4$ methods were GP-130, and a duplet of GP-90 and GP-80 which were poorly iodinated by lactoperoxidase/^{125}I. The galactose oxidase/NaB(^{3}H)$_4$ reaction was increased by between 4- and 10-fold and many additional glycoproteins were labelled after neuraminidase treatment, indicating that the cell surface galactose and *N*-acetylgalactosamine residues of glycoproteins are highly sialylated. In cell lines RT112 and RT4 there was prominent labelling of very high molecular weight sialogalactoconjugates that was not present in extracts of T24 and EJ.

Introduction

Tumour development and metastasis are accompanied by disturbances of the normal processes of intercellular recognition and adhesion and may involve changes in the composition and structure of cell surface glycoproteins and glycosaminoglycans [1–5]. Alterations in glycoproteins have been reported for leukemias [6], melanomas [7] and various mesenchymal tumour cell lines including virus-transformed fibroblast lines [8–10]. The common adult tumours are mostly of epithelial origin but relatively few studies have been involved with carcinoma cells and not many surface proteins of epithelial cells have been identified; very few have been purified and characterized [11–18]. The availability of cell lines RT112, RT4, T24 and EJ from transitional cell carcinomas allowed us to study the surface components of human bladder carcinomas. Previous studies of these lines have included chromosome distributions, tumorigenicity in nude mice, growth in agar [19–21], cell-mediated immunity [13,22–24], human lymphocyte antigen typing and isozyme analysis [25] and expression of oncogenes [26–32]. Two of these lines, RT112 and RT4, were derived from relatively well-differentiated carcinomas and grow

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Abbreviation: PMSF, phenylmethylsulphonyl fluoride.

in culture as cell islands, but fail to form colonies in agar. Cell line T24, derived from an anaplastic tumour, shows a tendency to proliferate as single cells in culture and forms colonies in agar. In this study the composition of exposed surface glycoproteins has been identified in these cell lines using three complementary radiolabelling methods: lactoperoxidase-catalyzed iodination, specific for tyrosine residues [33]; $NaIO_4$/ $NaB(^3H)_4$, specific for sialic acid residues [34]; and D-galactose oxidase/$NaB(^3H)_4$ including neuraminidase treatment, specific for D-galactose or *N*-acetylgalactosamine residues [35]. These data will be of use as a frame of reference for the identification of glycoproteins involved in epithelial tumour cell behaviour. The other widely distributed bladder cell line included in this study, EJ, has a morphology very similar to that of T24 and these two lines express a common oncogene [31,32]. In this report we show that T24 and EJ also have an almost identical profile of surface membrane proteins, consistent with the recent human lymphocyte antigen typing and isozyme studies which suggested that the EJ line used in these studies may be contaminated with cell line T24 [25].

Materials and Methods

Cell lines and culture. These cell lines were derived from three human bladder transitional cell carcinomas: the two lines RT112 and RT4 were established in this department by C.C. Rigby and L.M. Franks from two relatively well-differentiated tumours. Cell line T24, designated T24LMF in Ref. 25, was derived from a more anaplastic tumour [23] and was supplied by Dr. C.C. Rigby, Institute of Urology, London, U.K. Cell line EJ (also designated MGH U1) was supplied by Dr. J. Daly, Massachusetts General Hospital, Boston, U.S.A., and has been maintained in long-term culture in this laboratory. A recent analysis of the cell lines [25] has shown that cell lines RT112 and RT4 may be clearly distinguished from T24 and EJ by human lymphocyte antigen and/or isozyme patterns, but EJ and T24 are indistinguishable and probably derived from the same source. Cells were grown in E4 (Dulbecco's modified Eagle's medium) supplemented with 10% bovine serum and penicillin, streptomycin (100 units/ml) and kanomycin sulphate (100 μg/ml) in an atmosphere of 10% CO_2 in air, and cultures at confluence were passaged by resuspension with 0.06% trypsin/1 mM EDTA and split at a ratio of 1:5 (RT112 and RT4) or 1:10 (T24 and EJ). Cell cultures were routinely monitored by the method of Fogh and Fogh [36] for the prescence of *mycoplasma* which were not detected in RT112, RT4 and EJ but were present in the T24 cells.

Radioactive labelling of glycoproteins. The cell surface labelling methods were carried out on confluent cultures of cells in situ. Culture medium was removed and the cells were washed four times with Dulbecco's phosphate-buffered saline. Lactoperoxidase-catalyzed iodination was by the method of Tweto et al. [37] and was conducted at room temperature for 30 min. Carrier iodide and phenol red were omitted from the reaction medium, which contained 55 mM glucose, 10 mU/ml glucose oxidase (Sigma type II), 0.8 units/ml milk lactoperoxidase (Sigma Chemical Co. Ltd., Dorset, U.K.) and 50 μCi/ml ^{125}I(15 mCi/μg, Radiochemical Centre, Amersham, U.K.) when samples were for SDS-gel analysis, or 250 μCi/ml ^{125}I for labelling of cells for use in two-dimensional gels or autoradiography of cells. The externally exposed glycoproteins were also labelled by $NaB(^3H)_4$ reduction procedures of Gahmberg and Hakomori [35] and Gahmberg and Andersson [34]. Cell cultures were incubated at 37°C for 1 h in Hanks' balanced salts medium containing 5 mM glucose, with either 100 units/ml galactose oxidase (Sigma Chemical Co. Ltd., Dorset, U.K.) alone, or galactose oxidase plus 0.025 I.U. (12.5 units)/ml *Vibrio cholerae* neuraminidase (C.P. Laboratories Ltd., Bishops Stortford, U.K.). Other cultures were treated with ice-cold 0.5 mM $NaIO_4$ in phosphate-buffered saline for 15 min, then washed with buffered saline containing 25 mM glycerol. Cultures were washed twice with phosphate-buffered saline and were reduced with 0.5 mCi/ml $NaB(^3H)_4$ (28 Ci/mmol, Radiochemical Centre, Amersham, U.K.) for 15 min at ambient temperature. The $NaIO_4/NaB(^3H)_4$ procedure appears to be specific for sialic acid residues, as mild hydrolysis of labelled cell extracts in 0.5 M H_2SO_4 at 80°C for 1 h released 85% of the incorporated tritium. After these labelling procedures more than

95% of the cells remained viable as shown by trypan blue exclusion.

Gel electrophoresis. Cell samples for SDS-gel electrophoresis were resuspended in 'final sample buffer' [38] by brief sonication and run on 8–18% gradient or 10% polyacrylamide gels using the buffer system of Laemmli [38]. Gels were stained with Page blue 90 [39] and treated for fluorography by the method of Bonner and Laskey [40]. Fluorography and autoradiography were performed at −70°C using Kodak X-ray film, and densitometric scans and quantitation of the films were made using a Joyce-Loebl Chromoscan 3 microdensitometer. Protein mixtures (Bio-Rad Laboratories Ltd., Watford, U.K.) and [^{14}C]methylated protein mixture (Radiochemical Centre, Amersham, U.K.) were run as molecular weight standards: myosin (200 000), β-galactosidase (116 250), phosphorylase *b* (92 500), bovine serum albumin (67 000), ovalbumin (45 000), carbonic anhydrase (31 000), soybean trypsin inhibitor (21 500) and lysozyme (14 400). Cell samples for isoelectric focussing were suspended in 'lysis buffer' [41] containing 1 mM PMSF, and after 15 min incubation on ice samples were centrifuged for 5 s in a microfuge and the supernatant was stored at −20°C until electrophoresis (within 1 week). Samples were subjected to nonequilibrium isoelectric focussing for 1400 V h as described by O'Farrell [42] or to equilibrium isoelectric focussing at 400 V for 14 h then 800 V for 1 h [41]. The gel was equilibrated in 3% SDS, 0.375 M Tris-HCl (pH 8.8), 50 mM dithiothreitol [43] at room temperature for 15 min then run on 10% polyacrylamide slab gels, without a stacking gel, using the buffer system of Laemmli [39]. To determine the pH gradient after isoelectric focussing, gel sections (5 mm) of a parallel gel containing sample were incubated overnight with 1 ml distilled water at room temperature and the pH was measured.

Cell fixation and microscopy. Cell cultures of EJ and RT112 were prepared for autoradiography and microscopy as follows: lactoperoxidase iodination with ^{125}I as above, rinse twice in Hanks' with glucose, fix in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer pH 7.4 for 1 h at 4°C, wash repeatedly over 18–20 h in cold buffer containing 0.2 M sucrose, fix in 1% osmic acid for 1 h at room temperature (some specimens), dehydrate in methanols and through epoxipropane to embed in Araldite. Cell cultures were scraped and processed as pellets after dehydration in 70% methanol. Light-microscopy sections (0.5–1 μm) were coated with Ilford L4 emulsion, developed in D19 (Kodak) after exposure and examined unstained by phase microscopy. Thin sections for transmission electron microscopy autoradiography were mounted on nickel grids and coated with L4 emulsion using the loop method according to Williams [44]. Exposed grids were processed in D19 and sodium thiosulphite and post-stained with uranyl acetate and lead citrate. Grain distributions were analysed quantitatively from micrographs (×9800 × 3) by the method of Blackett and Parry [45].

Results

Plasma membrane glycoprotein subunits radiolabelled by the three surface-specific methods were resolved by SDS-electrophoresis under reducing conditions on gradient polyacrylamide gel (Figs. 1,

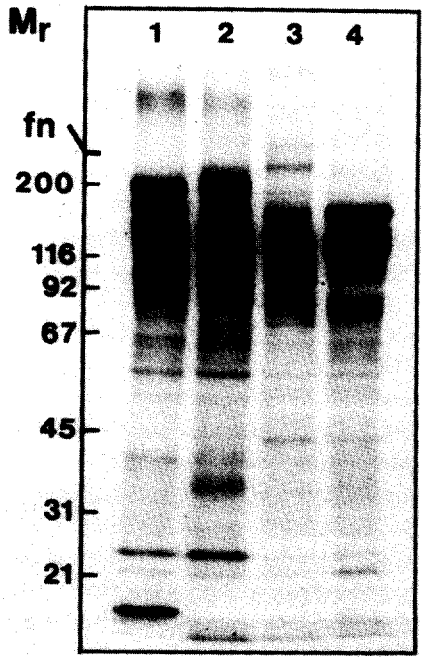

Fig. 1. One-dimensional electrophoresis of the lactoperoxidase/^{125}I-labelled surface proteins of cell lines RT112 (track 1), RT4 (track 2), T24 (track 3) and EJ (track 4). fn marks the position of fibroblast fibronectin.

3 and 4). Due to the complexity of the profiles evident in these figures evaluation has been restricted to those glycoproteins present in the higest quantities as determined by surface labelling and resolution by electrophoresis.

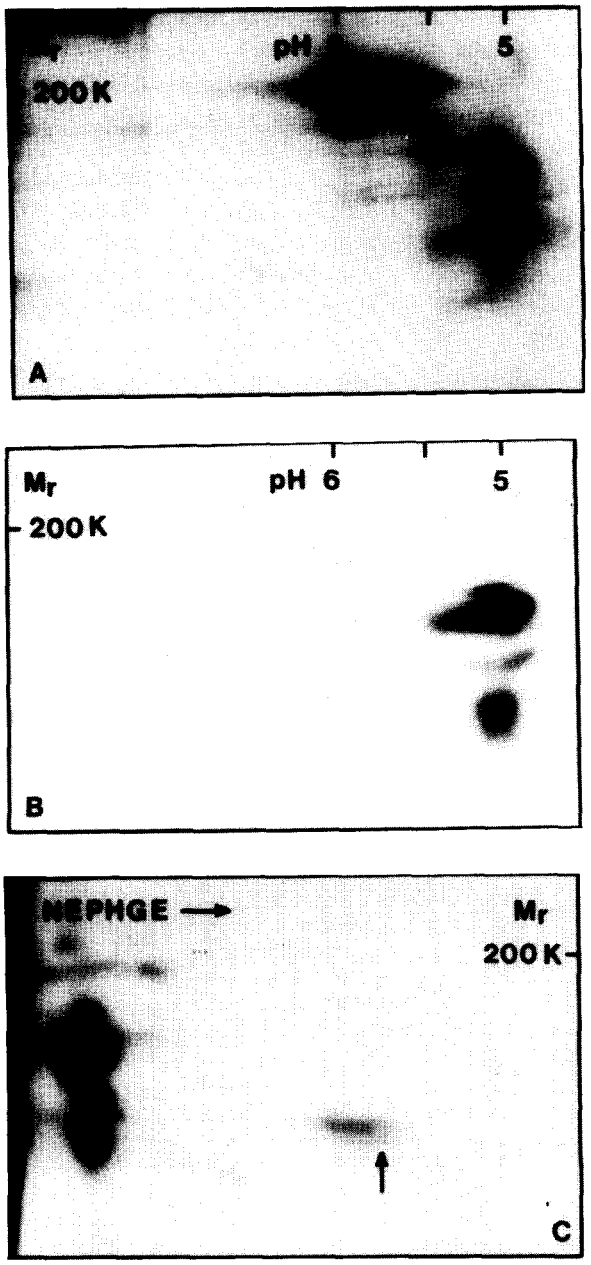

Fig. 2. Two-dimensional gel electrophoresis of the lacteroperoxidase/^{125}I-labelled surface proteins. Equilibrium isoelectric focussing of RT112 (panel A) and EJ (panel B), and nonequilibrium focussing of EJ (panel C). Glycoproteins in the molecular weight range 200000 to 100000 are shown.

Iodination with lactoperoxidase/^{125}I

Two control experiments gave indirect evidence that the iodination reaction was specific for cell surface components: (i) the reaction was virtually abolished by omitting lactoperoxidase and/or glucose oxidase from the incubation, and (ii) incubating the cells with the enzymes but without ^{125}I, followed by briefly washing, then incubating the cells with ^{125}I only, did not result in labelling. This experiment shows that the reaction was enzyme-dependent and any pinocytosis of active enzymes that may have occurred in these epithelial cells did not contribute to the iodination reaction to an appreciable extent. The cellular localization of iodinated material in EJ and RT112 was directly examined by autoradiography. Light microscopy showed intracellular activity. Transmission electron microscopy of EJ cells confirmed that, although grains were predominantly on the cell surface, intracellular grains were also present. Cross-scatter analysis of electron micrographs showed 75% of relative activity on the cell surface, 18% in the cytoplasm and 6% intranuclear (Table I). This indicates that some internalization of labelled surface proteins does occur during the reaction period, as has been reported in hepatoma cell lines [46,47]. Autoradiographs of cells also showed occasional unquantifiable extracellular hot spots, which were excluded from the analysis.

Fig. 1 shows that the most prominently iodinated bands were in the M_r 100000–200000

TABLE I

COMPUTED DISTRIBUTION OF RADIOACTIVE SOURCES BETWEEN VARIOUS CELLULAR COMPARTMENTS

These data (±S.E.) are derived from grain counts of EM autoradiographs, analysed according to Blackett and Parry [45]. EC, extracellular space.

Source	Grains/grid point	Relative activity (%)	Relative area (%)
EC	0.004±0.007	0.9±1.6	67.9±0.6
Cytoplasm	0.216±0.038	17.9±2.9	24.2±0.5
Nucleus	0.212±0.033	5.7±0.8	7.9±0.4
Cell surface	1.142±0.068	75.5±3.5	0 ±0

range, but there were a number of other labelled bands at lower molecular weight, including an M_r 25 000 band which was strongly labelled in RT112 and RT4 but was much less evident in T24 and EJ. To achieve greater resolution around the crowded higher molecular weight region of the gel the separation was repeated on two-dimensional gels using 10% polyacrylamide in the second dimension. Fig. 2A, B shows RT112 and EJ. The patterns for RT4 and T24 were identical to those for RT112 and EJ, respectively (not shown). The glycoproteins were resolved into seven major species which showed charge heterogeneity that was not seen in the total cell proteins stained with Page blue 90. A very striking difference between the lines was an iodinated protein of M_r 200 000/p*I* 5.5 to 6.0, which was present in RT112 and RT4 but absent from T24 and EJ. This iodoprotein had an apparent M_r of 200 000 in 12.5% and 7.5% polyacrylamide gels under both reduced and non-reduced conditions and so does not appear to contain large portions of carbohydrate that produce anomalous behaviour on SDS-electrophoresis [48]. This GP-200 migrated ahead of the fibronectin band of iodinated human fibroblast glycoproteins (arrow, Fig. 1). Five major iodinated proteins that were present in all four lines, although to different extents, were GP-175/p*I* 5.8–6.0; GP-155/p*I* 5.0–5.3, which in RT112 and RT4 was associated with a second species of p*I* 5.3–5.5 (see Fig. 2A); GP-145/p*I* 4.9–5.2; GP-130/p*I* 4.8–5.5 which showed marked streaking; and GP-110/p*I* 4.9–5.3. Another iodinated protein of M_r 110 000 was detected in both RT112 and EJ as a rapidly migrating species in nonequilibrium IEF gels and must have a p*I* of not less than 7.2 (arrow in Fig. 2C); RT4 and T24 were not examined by nonequilibrium IEF for a similar basic protein.

Sugar labelling with galactose oxidase / NaB(^{3}H)$_4$

The galactose oxidase/NaB(^{3}H)$_4$ method labels exposed glycoproteins with D-galactosyl and/or *N*-acetylgalactosaminyl residues at the nonreducing terminal of the carbohydrate moieties [35]. The iodinatable bands GP-200, GP-175, GP-155 and GP-145 were not detected by the galactose oxidase/NaB(^{3}H)$_4$ method, but by contrast GP-130 was the major band labelled in the four lines. In control experiments (no galactose oxidase present) faint labelling of a band at M_r 50 000 occurred in RT112 and RT4 (similar non-enzymatically labelled bands have been noted previously [7,49]). Treatment with galactose oxidase enhanced labelling of this M_r 50 000 band, and in all four cell lines other bands were seen at M_r 80 000 and 90 000 after long fluorographic exposure periods (Fig. 3).

Sugar labelling with neuraminidase-galactose oxidase / NaB(^{3}H)$_4$ and NaIO$_4$ / NaB(^{3}H)$_4$

Glycoproteins with their surface galactosyl/*N*-acetylgalactosaminyl residues masked by sialyl groups at the non-reducing end of the carbohydrate chain are generally not accessible to galactose oxidase [356]. These sialoglycoproteins may be labelled either in the terminal sialic acid group by mild oxidation with $NaIO_4$ and reduction with NaB(^{3}H)$_4$ [34], or the sialyl groups may be removed with neuraminidase and the penultimate galactosyl/*N*-acetylgalactosaminyl groups labelled by galactose oxidase/NaB(^{3}H)$_4$ [35]. In the four cell lines studied many galactoproteins were not labelled by galactose oxidase/NaB(^{3}H)$_4$ unless neuraminidase was present. The galactose oxidase/NaB(^{3}H)$_4$ reaction was increased by approx. 10-fold in RT112 and RT4 and by approx. 4-fold in T24 and EJ after neuraminidase treatment, indicating that the surface galactosyl/*N*-

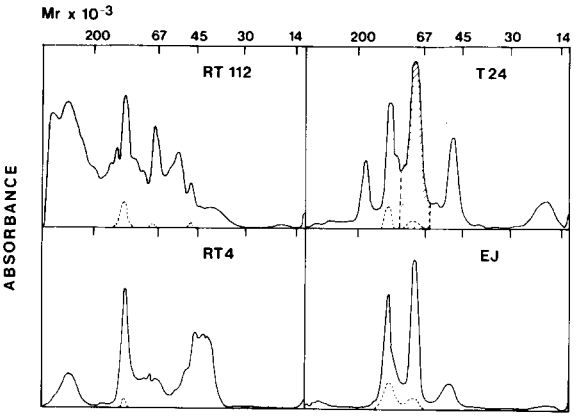

Fig. 3. Densitometric tracings of fluorographs of glycoproteins labelled by the galactose oxidase/NaB(^{3}H)$_4$ method and separated by electrophoresis on gradient polyacrylamide gels. With (continuous line) or without (dashed line) neuraminidase treatment. The shaded areas are referred to in the text.

acetylgalactosaminyl residues have a very high degree of sialylation. Figs. 3 and 4 show that essentially the same profiles of sialoglycoproteins were labelled within each cell line by the neuraminidase-galactose oxidase/$NaB(^3H)_4$ and $NaIO_4$/$NaB(^3H)_4$ methods, with small quantitative differences.

There was prominent labelling in RT112 and RT4 by neuraminidase-galactose oxidase/$NaB(^3H)_4$ and $NaIO_4/NaB(^3H)_4$ of very high molecular weight material, but these sialogalactoconjugates were not seen in T24 or EJ. In RT112 labelled by $NaIO_4/NaB(^3H)_4$ this material accounted for 48% of the label resolved on gel electrophoresis as estimated from the densitometric tracings (see shaded area, Fig. 4).

The iodinated bands GP-200, GP-175, GP-155 and GP-145 were moderately labelled by the $NaIO_4/NaB(^3H)_4$ and neuraminidase-galactose oxidase/$NaB(^3H)_4$ methods, suggesting that these are minor sialogalactoproteins of the cell surface (Fig. 5). A complex pattern of sialoglycoproteins was labelled in the M_r 150000–70000 region in RT112 and RT4 by the two labelling methods, but in T24 four major peaks were labelled and two

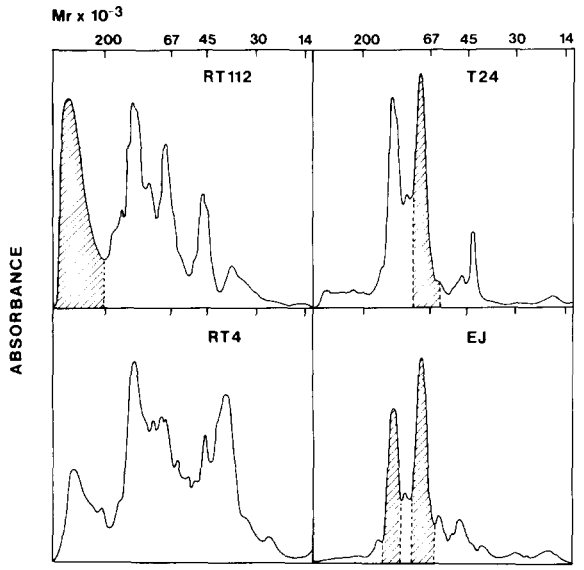

Fig. 4. Densitometric tracings of fluorographs of glycoproteins labelled by the $NaIO_4/NaB(^3H)_4$ method and separated by electrophoresis on gradient polyacrylamide gels. The shaded areas are referred to in the text.

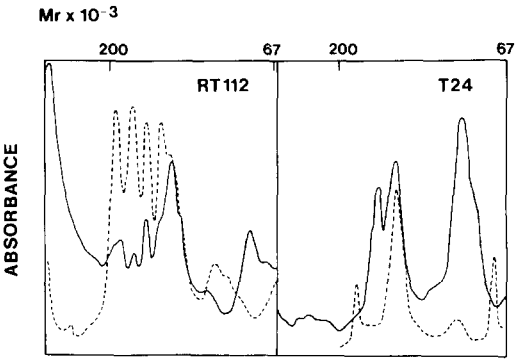

Fig. 5. Densitometric tracings of glycoproteins labelled by the lactoperoxidase/^{125}I (dashed line) or $NaIO_4/NaB(^3H)_4$ (continuous line) methods and separated by electrophoresis on 10% polyacrylamide gel.

major peaks were seen in EJ (Figs. 3 and 4). The major sialogalactoproteins labelled by the neuraminidase-galactose oxidase/$NaB(^3H)_4$ and $NaIO_4/NaB(^3H)_4$ in all four lines were GP-130 and the GP-80 to GP-90 region: these bands were slightly displaced due to increased electrophoretic mobility after removal of sialic acid residues in the enzyme-catalyzed method. The charge heterogeneity seen in isoelectric focussing of iodinated GP-130 (Fig. 2A, B) may reflect different degrees of sialylation of the glycoprotein [50]. In EJ the GP-130 band accounted for 30% of the radioactivity incorporated into glycoproteins by either $NaIO_4$/$NaB(^3H)_4$ or neuraminidase-galactose oxidase/$NaB(^3H)_4$ methods. The GP-80–GP-90 region was also very prominent in T24 and EJ, containing 35% of the residues which were labelled by either method (see shaded areas in Figs. 3 and 4).

Cell line RT4 showed prominent labelling at M_r 40000 by both sialoglycoprotein labelling techniques. In each of the $NaIO_4/NaB(^3H)_4$ and neuraminidase-galactose oxidase/$NaB(^3H)_4$ methods, some radioactivity (presumably lipids) ran in front of the bromophenol blue dyefront.

Proteins distinguishing T24 from EJ

Cell line T24 could be distinguished from EJ by the presence in T24 of a sialogalactoprotein of apparent M_r 150000 in 10% polyacrylamide (first peak of continuous line in Fig. 5, T24) and 8–18% gradient gels (Fig. 4) after $NaIO_4/NaB(^3H)_4$ la-

belling. This sialogalactoprotein was not labelled in cells treated with galactose oxidase/$NaB(^3H)_4$ but was labelled by neuraminidase-galactose oxidase/$NaB(^3H)_4$ and showed decreased electrophoretic mobility after desialylation, with apparent M_r 200 000 in 10% gels (not shown) and an apparent M_r 175 000 in 8–18% gradient gels (this band was very prominent compared to the amount of label associated with GP-175 in 10% gels) (see Fig. 3). T24 also showed a more prominent band at M_r 50 000 by both $NaIO_4/NaB(^3H)_4$ and the neuraminidase-galactose oxidase/$NaB(^3H)_4$ procedures. Detailed comparison of the total cellular protein profile of the two lines in one-dimensional gels indicated no quantitative differences between T24 and EJ that could be detected by Page blue 90 staining, whereas cell lines RT112 and RT4 could be clearly distinguished from T24 and EJ by the protein profile, particularly in the cytoskeletal protein region, M_r 40 000–65 000 (not shown).

Discussion

This comparison of the exposed glycoproteins of bladder carcinoma cell lines has shown that the pattern of major glycoproteins identified by three cell-surface labelling methods was similar, but nevertheless distinctive, in cell lines RT112, RT4 and T24. These results are complementary to studies of bladder carcinoma surface antigens involved in cell-mediated immunity [13,22–24] and the differentiation of bladder epithelium [51–53] and also to previous studies of other tumour cell lines [6–18,46,47,54]. As the profiles of total cellular proteins and surface glycoproteins after gel electrophoresis are distinctive for individual cell lines, it has been proposed that these profiles may be used to distinguish between cell lines [7,13]. The fourth cell line included in this study, EJ, showed a profile of surface labelled glycoproteins which is qualitatively and quantitatively almost identical to that of T24, adding further evidence to the suggestion that this widely distributed EJ line has become contaminated with the T24 cell line or a subline of T24 [25]. The sialogalactoprotein present in T24 but not EJ (see Results) may represent only a clonal variation between otherwise identical cell lines, similar to that found in chromosome pattern and behaviour between different clones of EJ cells [55] or it may be due to the presence of *mycoplasma* in the T24 cells.

A complex profile (more than 30) of polypeptide components were labelled by lactoperoxidase-catalyzed iodination and two sialoglycoprotein-labelling methods ($NaIO_4/NaB(^3H)_4$ and neuraminidase-galactose oxidase/$NaB(^3H)_4$). Most if not all of the iodinated glycoproteins were sialoglycoproteins and were labelled by the sugar-labelling methods (as was seen with hepatoma cell lines [46]). This study did not aim at detailed structural analysis of the surface-labelled glycoproteins, but the properties of the major labelled glycoproteins which may be inferred from the methods used are summarized in Table II. The most heavily iodinated glycoproteins which were evaluated in this study were of high molecular weight (M_r 100 000–200 000) and rather acidic isoelectric point. One prominently iodinated glycoprotein, designated GP-200, was present in well-differentiated lines RT112 and RT4 but absent from T24 (and EJ): GP-200 is likely to be an M_r 200 000 tumour-associated antigen which has recently been demonstrated in the urine of patients with bladder transitional cell carcinoma [56]. GP-200 is unlikely to be fibronectin has it did not cochromatograph with the fibroblast fibronectin band in SDS-electrophoresis and these cell lines were completely negative by immunofluorescence both for surface or intracellular fibronectin [53]. It is also improbable that GP-200 is carcinoembryonic antigen (CEA), as GP-200 was poorly labelled with the $NaIO_4/NaB(^3H)_4$ method whereas CEA is highly sialylated and has a low p*I* value [11]. GP-110 has previously been shown to be a surface antigen which is present on the membrane of T24 and other human bladder lines as well as normal bladder epithelium [13] and other human tumour lines [57].

The glycoproteins of these bladder carcinoma cell lines were poorly reactive to galactose oxidase/$NaB(^3H)_4$ unles the cells were desialylated with neuraminidase, as has also been found with melanoma cell lines [7] and H-35 and Novikoff hepatoma cell lines [46,54]. Although increased cell surface sialic acid has been implicated in masking antigenic determinants of neoplastic cells (see Ref. 2), the relevance to malignancy of

TABLE II

SOME PROPERTIES OF MAJOR GLYCOPROTEINS

−, not detected in extended fluorograph exposure; +, detected in extended fluorograph exposure; + +, readily detected in short fluorograph exposure. Sia, sialoglycoprotein.

Properties	Structural interpretation	GP-200	Acidic	GP-175 Gp-175 GP-145 GP-110	GP-130 GP-90 GP-80
Labelled by galactose oxidase/ $NaB(^3H)_4$	Contains a non-reducing Gal accessible oxidase on intact cell	−		−	+
Labelled by $NaIO_4/NaB(^3H)_4$	*R*-Sia	+		+	+ +
Labelled by galactose oxidase/ $NaB(^3H)_4$ after neuraminidase	*R*-(DGal-or NAcGal)-Sia with terminal Sia residues accessible to neuraminidase	+		+	+ +
Labelled by lactoperoxidase/^{125}I	Has accessible tyrosine	+ +	GP-175 GP-155 GP-145 GP-110	+ + +	GP-130 + + GP-90 GP-80 −
Present in cell lines		RT112 and RT4 only		All	All

the high degree of sialylation of the glycoproteins of cultured cels is not clear. GP-130 and the duplet of proteins at GP-90 and GP-80, the proteins with exposed terminal galactose of *N*-acetylgalactosaminyl residues, were also the major sialoglycoprotein peaks common to all four lines after $NaIO_4/NaB(^3H)_4$ labelling. GP-130 appears to be closely related to a major sialogalactoprotein, Gal-1Pa, of AS30D hepatocarcinoma cells, on the basis of similar reactivity to galactose oxidase/ $NaB(^3H)_4$ and to lactoperoxidase-catalyzed iodination, similar p*I* values and apparent molecular weight in SDS-electrophoresis, and susceptibility to desialylation by neiuraminidase [49]. The identities of GP-90 and GP-80 are not known, but M_r 90000 sialoglycoproteins have been reported on human melanoma cells [7] and HeLa S3 cells [47], and Novikoff hepotoma cells [54], and GP-80 may be related to a major sialoglycoprotein of M_r 77000 found in urine [58]. The two sialoglycoprotein-labelling methods also showed differences between the lines which were not detected by iodination: (i) the sialoglycoprotein profile in the M_r 80000–200000 region was complex in RT112 and RT4 (at least 11 discrete bands were visible on the original fluorograph film), whereas only two to four sialoglycoproteins were identified in T24 and EJ, and (ii) high concentrations of glycosaminoglycans were present at the surface of the two well-differentiated cell lines in culture, as up to half of the labelled sialyl residues in RT112 and RT4 were in very high molecular weight sialogalactoconjugates which were not evident in labelled extracts from T24 and EJ (Figs. 3 and 4). This high molecular weight material may also contain the recently characterized tumour antigen Ca, a sialoproteoglycan dimer of M_r 390000 and 350000 which is expressed in the majority of malignant tumours including transitional cell carcinomas and which is also found in the normal transitional epithelium of bladder [17,18]. Tumour antigen Ca has been demonstrated in cell line RT112 but the other bladder carcinoma lines were not studied [17,18].

The objective of this study has been limited to the identification of cell surface glycoproteins of transitional cell carcinoma lines. The glycoproteins of other human tumour cells have recently been described using similar methods [7,11–14,16,47] and with further studies it should be possible to build up a catalogue of the major glycoprotein species of various types of tumour. The signifi-

cance of the glycoprotein and glycosaminoglycan similarities and differences seen in this study are not clear, as the specific physiological function of each of the glyconjugates is unknown. Partial purification and the production of monoclonal antibody probes to individual glycoproteins will make it possible (i) to study the distribution of these glycoproteins in other types of epithelia, and (ii) to determine whether the differences between the lines seen in this study reflect glycoprotein changes which are related to differentiation of bladder epithelium, and (iii) to study the function of these glycoconjugates in determining the ordered cellular structure of bladder epithelium and the breakdown of that structure in transitional cell carcinoma.

Note added in proof (Received June 8th, 1983)

Franke [59] has recently reported two major glycoproteins, putatively associated with desmosomes, which have molecular weights and isolectric points very similar to those of the GP-130 found in this study.

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References

1 Loewenstein, W.R. (1979) Biochim. Biophys. Acta 560, 1–65
2 Nicolson, G.L. (1976) Biochim. Biophys. Acta 458, 1–72
3 Yamada, K.M. and Pouyssegur, J. (1978) Biochimie 60, 1221–1233
4 Warren, L., Buck, C.A. and Tuszynski, G.P. (1978) Biochim. Biophys. Acta 516, 97–127
5 Hynes, R.O. (1976) Biochim. Biophys. Acta 458, 73–107
6 Blackstock, R. and Humphrey, G.B. (1982) in Methods in Cancer Research, Vol. 19 (Busch, H. and Yoeman, L.C., eds.), pp. 3–51, Academic Press, New York
7 Lloyd, K.O., Travassos, L.R., Takahashi, T. and Old, L.J. (1979) J. Natl. Cancer Inst. 63, 623–634
8 Baker, S.R., Blithe, D.L., Buck, C.A. and Warren, L. (1980) J. Biol. Chem. 255, 8719–8728
9 Dubbelman, T.M. and Yamada, K.M. (1982) Biochim. Biophys. Acta 693, 177–187
10 Winterbourne, D.J. and Mora, P.T. (1981) J. Biol. Chem. 256, 4310–4320
11 Tsao, D. and Kim, Y.S. (1978) J. Biol. Chem. 253, 2271–2278
12 Smith, H.S., Riggs, J.L. and Mosesson, M.W. (1979) Cancer Res. 39, 4138–4144
13 Schneider, M.U., Paulie, S., Troye, M. and Perlmann, P. (1980) Int. J. Cancer 26, 193–202
14 Kim, Y.S., Tsao, D., Hicks, J. and McIntyre, L.J. (1981) Cancer 47, 1590–1596
15 Iozzo, R.V. and Wight, T.N. (1982) J. Biol. Chem. 257, 11135–11144
16 Ogata, S-I., Ueda, R. and Lloyd, K.O. (1981) Proc. Natl. Acad. Sci. U.S.A. 78, 770–774
17 Ashall, F., Bramwell, M.E. and Harris, H. (1982) Lancet ii, 1–6
18 McGee, J.O'D., Woods, J.C., Ashall, F., Bramwell, M.E. and Harris, H. (1982) Lancet ii, 7–10
19 Marshall, C.J., Franks, L.M. and Carbonell, A.W. (1977) J. Natl. Cancer Inst. 58, 1743–1751
20 Hastings, R.J. and Franks, L.M. (1981) Int. J. Cancer 27, 15–21
21 Rigby, C.C. and Franks, L.M. (1970) Br. J. Cancer 24, 746–754
22 Schneider, M.U., Troye, M., Paulie, S. and Perlmann, P. (1980) Int. J. Cancer 26, 185–192
23 Bubenik, J., Baresova, M., Viklicky, V., Jakoubkova, J., Sainerova, H. and Donner, J. (1973) Int. J. Cancer 11, 765–773
24 O'Toole, C., Price, Z.N., Ohnuki, Y. and Unsgaard, B. (1978) Br. J. Cancer 38, 64–76
25 O'Toole, C., Povey, S., Hepburn, P. and Franks, L.M. (1983) Nature 301, 429–430
26 Der, C.J., Krontiris, T.C. and Cooper, G.M. (1982) Proc. Natl. Acad. Sci. U.S.A. 79, 3637–3640
27 Goldfarb, M., Shimizu, K., Perucho, M. and Wigler, M. (1982) Nature 296, 404–409
28 Parada, L.F., Tabin, C.J., Shih, C. and Weinberg, R.A. (1982) Nature 297, 474–478
29 Perucho, M., Goldfarb, M., Shimizu, K., Lama, C., Fogh, J. and Wigler, M. (1981) Cell 27, 467–476
30 Shih, C. and Weinberg, R.A. (1982) Cell 29, 161–169
31 Reddy, E.P., Reynolds, R.K., Santos, E. and Barbacid, M. (1982) Nature 300, 149–152
32 Tabin, C.J., Bradley, S.M., Bragmann, C.I., Weinberg, R.A., Papageorge, A.G., Scolnick, E.M., Dhar, R., Lowy, D.R. and Chang, E.H. (1982) Nature 300, 143–149
33 Hynes, R.O. (1973) Proc. Natl. Acad. Sci. U.S.A. 70, 3170–3174
34 Gahmberg, C.G. and Andersson, L.C. (1977) J. Biol. Chem. 252, 5888–5894
35 Gahmberg, C.G. and Hakomori, S. (1973) J. Biol. Chem., 248, 4311–4317
36 Fogh, J. and Fogh, H. (1964) Proc. Soc. Exp. Biol. Med. 117, 899–901

37 Tweto, J., Friedman, E. and Doyle, D. (1976) J. Supramol. Struct. 4, 141–159
38 Laemmli, U.K. (1970) Nature 227, 680–685
39 Reisner, A.H., Nemes, P. and Bucholtz, C. (1975) Anal. Biochem. 64, 509–516
40 Bonner, W.M. and Laskey, R.A. (1974) Eur. J. Biochem. 46, 83–88
41 O'Farrell, P.H. (1975) J. Biol. Chem. 250, 4007–4021
42 O'Farrell, P.Z., Goodman, H.M. and O'Farrell, P.H. (1977) Cell 12, 1133–1142
43 Garrels, J.I. (1979) J. Biol. Chem. 254, 7961–7977
44 Williams, M.A. (1977) in Practical Methods in Electron Microscopy (Glauert, A.M., ed.), Vol. 6, pp. 77–163, Elsevier/North-Holland, Amsterdam
45 Blackett, N.M. and Parry, D.M. (1977) J. Histochem. Cytochem. 25, 206–214
46 Baumann, H. and Doyle, D. (1978) J. Biol. Chem. 253, 4408–4418
47 Fishman, J.B. and Cook, J.S. (1982) J. Biol. Chem. 257, 8122–8129
48 Segrest, J.P. and Jackson, R.L. (1972) Methods Enzymol. 28B, 54–63
49 Glenney, J.R., Kaulfus, P.J., McIntyre, B.W. and Walborg, E.F. (1980) Cancer Res. 40, 2853–2859
50 Litin, B.S. and Grimes, W.J. (1979) Cancer Res. 39, 2595–2603
51 Nathrath, W.B.J., Detheridge, F. and Franks, L.M. (1979) J. Natl. Cancer Inst. 63, 1322–1330
52 Nathrath, W.B.J. and Franks, L.M. (1981) J. Urol. 126, 77–80
53 Trejdosiewicz, L.K., Smolira, M.A., Hodges, G.M., Goodman, S.L. and Livingston, D.C. (1981) J. Microsc. 123, 227–236
54 Glenney, J.R., Allison, J.P., Hixson, D.C. and Walborg, E.F. (1979) J. Biol. Chem. 254, 9247–9253
55 Hastings, R.J. and Franks, L.M. (1983) Br. J. Cancer 47, 233–244
56 Defazio, S.R., Gozzo, J.J. and Monaco, A.P. (1982) Cancer Res. 42, 2913–2917
57 Ogata, I. and Lloyd, K.O. (1982) Arch. Biochem. Biophys. 217, 665–673
58 Berggard, I., Ekstrom, E., Lisowska, E. and Lundblad, A. (1981) Eur. J. Biochem. 115, 183–188
59 Franke, W.W. (1982) Biol. Cell 45, 435