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Heterogeneity of Glycoprotein Synthesis in Human Tumor Cell Lines*

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Abstract—*In order to screen human tumor cells for putative cell surface marker molecules, the glycoprotein composition of* in vitro *cultivated human tumor cell lines of different origin* (12 *carcinomas, one neuroblastoma, one melanoma and one sarcoma) was analyzed by metabolically labelling the cells with* [^{3}H]*galactose,* [^{3}H]*mannose and* [^{3}H]*fucose and subsequently separating the labelled material by SDS–PAGE. The cell lines expressed their specific glycoprotein patterns. Strongly glycosylated proteins of apparent mol. wt* 40–45 kD, 60–62 kD, 80–82 kD *and* 90–92 kD *were shared by nearly all carcinoma cell lines studied. Apart from these glycoprotein clusters, a great diversity was observed between tumor cell lines derived from the same organ. Three bladder carcinoma cell lines had a* 112–114 kD *glycoprotein in common. Glycoprotein expression of these cell lines remained constant during* 1 yr *of* in vitro *culture. Hence, these glycoprotein patterns seem to be useful for monitoring the phenotypic stability of cell lines. A sarcoma cell line was deficient in incorporating fucose and showed strikingly different glycoprotein patterns compared to the other cell lines studied. The metabolic labelling procedure revealed a wide phenotypic heterogeneity of the human carcinoma cell lines concerning glycoprotein synthesis. This method contributes another parameter to map the major glycoprotein species of various types of carcinomas.*

INTRODUCTION

THE DETECTION of various cell surface markers both by biochemical methods and by application of monoclonal antibodies has facilitated the classification and diagnosis of human tumors traditionally based on histomorphological criteria. Thus, antigens of glycoprotein nature expressed on human leukemic cells [1] and on tumor cells of epithelial origin [2] have recently been described. So far, the existence of a tumor-specific antigen could not clearly be shown. In most cases the respective antigen was also detected on the normal corresponding cells. A comparison of glycoprotein patterns of human carcinoma cell lines could prove useful in a systematic search for marker antigens uniquely expressed by one tumor type. Up to now, mapping of glycoprotein patterns has been performed with certain types of human tumors such as leukemias [3], melanomas [4], colon carcinomas [5] and lung carcinomas [6]. Due to different methodological approaches a direct comparison of these glycoprotein patterns is difficult.

In this study the glycoprotein composition of a large variety of human tumor cell lines was analyzed by metabolically labelling the cells with tritiated carbohydrate precursors and subsequently separating the glycoproteins by SDS–polyacrylamide gel electrophoresis. This sensitive method enables the characterization of glycoprotein expression concerning glycosylation dynamics. As a prerequisite for *in vitro* studies of this kind we also investigated the stability of marker expression under standard culture conditions.

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MATERIALS AND METHODS

Tumor cell lines and culture conditions

The following *in vitro* passaged human tumor lines were used in the experiments: a large cell lung carcinoma cell line, ChaGo [7]; a small cell lung carcinoma cell line, oat-75 [8]; a squamous cell lung carcinoma cell line, SK-LC-LL [9]; a cell line derived from a lymph node metastasis of an adeno/squamous cell lung carcinoma, L26l, kindly provided by Dr. Sonka, German Cancer Center, Heidelberg; two colon carcinoma cell lines, HT-29 [10] and WiDr [9]; a larynx carcinoma cell line, HEp-2 [9]; a cervix carcinoma cell line, ME-180 [9]; a mamma carcinoma cell line, AlAb [9]; three bladder carcinoma cell lines, J82 [11], RT 4 [12], T24 [12], all kindly supplied by Dr. C. O'Toole, Department of Pathology, Addenbrooke's Hospital, Cambridge, UK; a melanoma cell line, MML II, kindly obtained from Dr. Tilgen, Dermatology Department, University Clinics, Heidelberg; a sarcoma cell line, P5A; and a neuroblastoma cell line,

SK-N-SH [13]. All cell lines were adherent and were cultured in RPMI 1640 medium (Gibco-Biocult, Glasgow, U.K.) supplemented with 10% fetal calf serum. The cell cultures were routinely screened for the absence of mycoplasms [14]. Contaminated cell cultures were freed from mycoplasms by treatment with tiamutin and minocyclin, as described by Schmidt and Erfle [15].

Incorporation assays

For determination of cell proliferation 1 μCi of [^{3}H]thymidine (23.6 Ci/mmol) adjusted to 5 μM with unlabelled thymidine, for determination of protein synthesis 1 μCi of [^{3}H]leucine (60 Ci/mmol) and for determination of glycoconjugate synthesis 2 μCi of [^{3}H]galactose (15 Ci/mmol) and 2 μCi of [^{3}H]fucose (24 Ci/mmol) adjusted to a final concentration of 20 μM with the respective unlabelled carbohydrates were added in 20-μl portions to 2 × 10^5 cells/well (flat-bottomed; Greiner, Nürtingen, F.R.G.) at the beginning of the assays. All radioactive substances were supplied by Amersham & Buchler, Braunschweig, F.R.G. The cells were harvested after 24 hr by acid precipitation with 0.4 M perchloric acid on glass fiber filters using a skatron multiple cell culture harvester (Flow, Bonn, F.R.G.). Samples were counted in emulsifier scintillator solution in a Packard liquid scintillation counter. All incorporation experiments were carried out in triplicate.

Incorporation of ^{3}H-labelled sugars into tumor cells, SDS-PAGE and autoradiography

For metabolic labelling of glycoproteins exponentially growing tumor cells were seeded into flat bottom microtiter plates at a concentration of 2 × 10^5 cells/well. To avoid alterations of cell surface molecules by trypsination the adherent cells were mechanically scraped off the culture plates. Viability of the cell cultures thus treated was over 90% as measured by the Trypan blue exclusion test. Cells were labelled with 20 μCi [D-1-^{3}H]galactose/well, 20 μCi [D-2-^{3}H]mannose/well (9.3 Ci/mmol) and 20 μCi of [L-6-^{3}H]fucose/well for 16 hr under regular culture conditions. Cells were harvested, then triplicates were pooled and washed twice with phosphate-buffered saline. Cell-bound proteins were solubilized in Triton X-100 extraction buffer [16]. After centrifugation in an Eppendorf centrifuge for 15 min at 4°C supernatants were mixed with 0.5 M Tris–HCl buffer, pH 6.8, containing 2% SDS (v/v) and were heated in a boiling water bath for 3 min. Proteins were precipitated with cold acetone and the precipitate was solubilized in disintegration buffer [17] containing 2% SDS and 2% 2-ME. Samples were applied to polyacrylamide slab gel electrophoresis in 8% gels using a discontinuous buffer system according to Laemmli [17]. Myosin (mol. wt 200,000), phosphorylase B (192,500), bovine serum albumin (69,000), ovalbumin (46,000) and carbonic anhydrase (30,000), all methyl-^{14}C-labelled, were used as reference proteins. Slab gels were fixed overnight in an isopropanol/acetic acid/water mixture (2.5/1/6.5, v/v/v) and then treated with a DMSO solution containing 2.5-diphenyloxazol according to Bonner and Laskey [18]. RPX-O-mat X-ray films (Kodak, Rochester, NY, U.S.A.) were exposed to the dried gels for 14–21 days. For comparative evaluation autoradiograms were scanned with a Chromoscan 3 (Joyce-Loebl, Düsseldorf, F.R.G.).

RESULTS

Incorporation of precursors

In order to determine whether the glycosylation rate of proteins was correlated with protein synthesis and proliferation, incorporation of radioactively labelled precursors into tumor cell lines was measured. When comparing the amount of incorporated precursors into the cell lines (Table 1), DNA- and protein synthesis always showed similar dynamics while the incorporation of carbohydrates showed different dynamics. These results indicate that glycosylation is not directly dependent on the degree of protein synthesis and proliferation. Glycoprotein patterns obtained by the metabolic labelling method do not necesarily reflect turnover rates of proteins but rather point to cell line specific glycosylation patterns of surface expressed proteins.

Glycoprotein patterns of tumor cell lines

Glycoprotein (gp) patterns of 15 human tumor cell lines were obtained by metabolic labelling with radioactive carbohydrates. We chose [^{3}H]galactose, [^{3}H]mannose and [^{3}H]fucose because these sugars represent some of the major constituents of the carbohydrate moieties of glycoproteins. According to the migration of the calibration proteins, molecular weights could be determined in the range of 35 to about 200 kD, using 8% gels throughout the experiments.

In general, all carcinoma cell lines shared gps clustered at apparent mol. wts of 40–45 kD, 60–62 kD, 80–82 kD and 90–92 kD. Individual glycoprotein patterns of all cell lines studied will be described comparatively according to the origin of the respective tumor lines.

Labelling patterns of lung carcinoma cell lines

The four cell lines studied represent the major types of lung cancer: squamous cell carcinoma (SK-LC-LL), adeno carcinoma (L261), large cell carcinoma (ChaGo) and small cell carcinoma (oat-75). Glycoprotein patterns of these cell lines are

Table 1. *Incorporation of* ^{3}H*-labelled precursors into carcinoma cell lines*

Tumor cell line	[^{3}H]Thymidine	[^{3}H]Leucine	[^{3}H]Galactose	[^{3}H]Fucose
SK-LC-LL	7800*	15500	42800	12300
ChaGo	29600	29600	45800	13400
L26 1	3000	13600	15500	1900
WiDr	12900	19600	53900	15000
Hep-2	16400	18800	32600	8300
AlAb	17500	23100	30600	3600

* cpm/culture after 24 hr pulse labelling.

shown in Fig. 1.

Cell line SK-LC-LL was characterized by two strongly [^{3}H]galactose-labelled, high-molecular-weight bands (185 kD, 200 kD). In comparison to the other lung carcinoma cell lines, SK-LC-LL displayed a strikingly high number of gps only labelled with [^{3}H]mannose, which points to the presence of 'high-mannose-type' gps (39, 42, 47, 54, 60, 92, 111, 121, 136, 163 kD). Some bands were only visible with [^{3}H]fucose label (40, 44, 49, 55, 75, 86, 94 kD). Some bands were labelled both with [^{3}H]mannose and [^{3}H]fucose. Tumor cell line L26 1 had its specific gps labelled with all three markers (hereafter described as 'completely labelled') (58, 72, 97, 112, 124, 132, 154 kD). High-molecular-weight gps, above 154 kD, were absent in this cell line.

Completely labelled gps of cell line ChaGo could be identified at mol. wts 104, 122, 134, 177 kD. Highly fucosylated gps were at the position of 42, 48, 50, 150, 198 kD. Small cell carcinoma line oat-75 displayed its completely labelled gps markers at mol. wt 50, 78, 180 kD, a [^{3}H]galactose-labelled gp at 150 kD and some [^{3}H]mannose-labelled gps at 68, 72, 84, 94 kD. Glycoprotein bands indicating a relationship of these lung-derived tumors could not be detected.

Labelling patterns of colon carcinomas

Both colon cell lines investigated showed different gp patterns (Fig. 2). These cell lines had in common only a cluster of fucosylated gps in the range of approximately 53–62 kD.

Cell line WiDr was characterized by two strongly [^{3}H]galactose-labelled bands (146, 207 kD), two [^{3}H]mannose-labelled gps at 102 and 134 kD and some bands with [^{3}H]fucose label (48, 87, 198 kD). The other colon cell line, HT-28, could be defined by a specific, completely labelled gp at 106 kD, [^{3}H]mannose-labelled bands at 116, 138, 142 kD and a [^{3}H]fucose-labelled band at 126 kD.

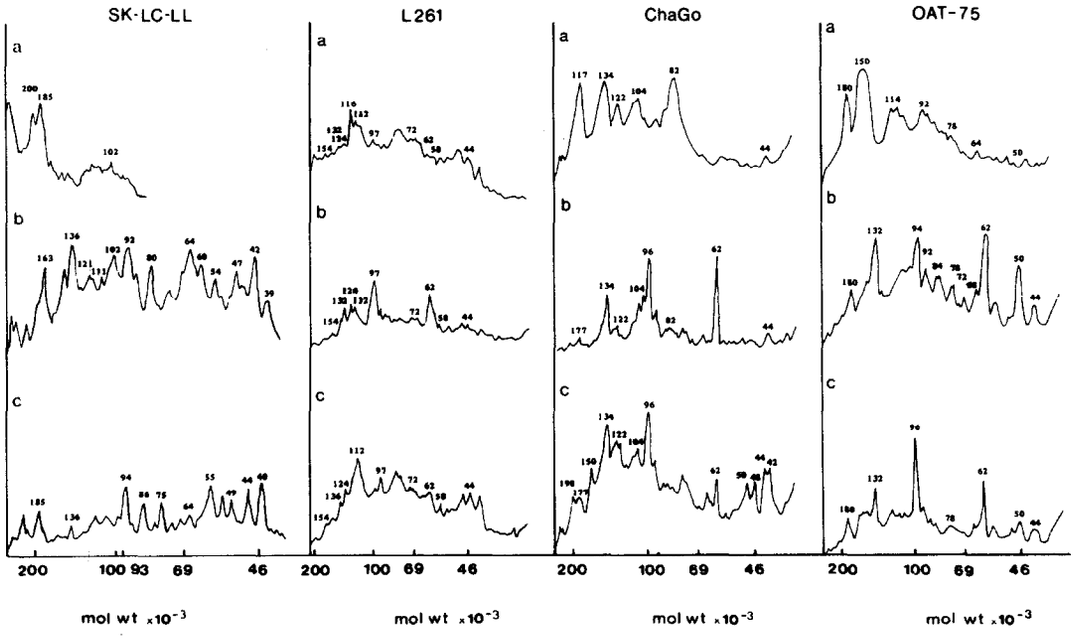

Fig. 1. *Scanning profiles of fluorographs of* ^{3}H*-labelled glycoproteins derived from human lung carcinoma cell lines.* (*a*) [^{3}H]*Galactose label,* (*b*) [^{3}H]*mannose label;* (*c*) [^{3}H]*fucose label.*

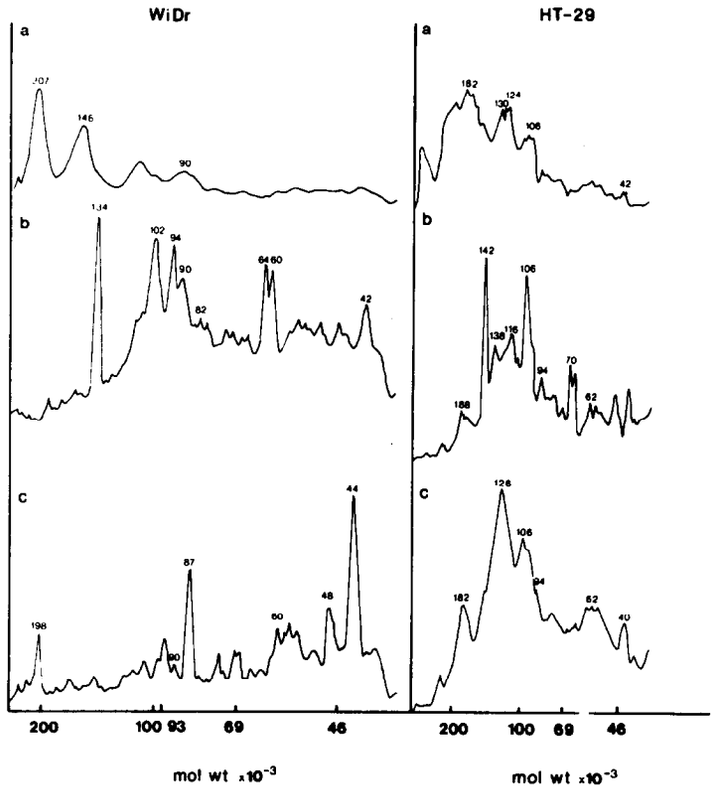

Fig. 2. Scanning profiles of fluorographs of ^{3}H-labelled glycoproteins derived from human colon carcinoma cell lines. (a) [^{3}H]Galactose label; (b) [^{3}H]mannose label; (c) [^{3}H]fucose label.

Labelling patterns of bladder carcinomas

Three bladder carcinoma cell lines taken for this study have been described by their morphology, HLA-class I phenotypes and isoenzymes [11, 12]. Glycoprotein patterns of the bladder carcinoma cell lines are presented in Fig. 3. Despite their phenotypic differences, these cell lines shared a marker gp labelled strongly with all three radioactive sugars with a mol. wt of 112–114 kD. A gp with similar characteristics was only identified in L261 cells. The well-differentiated cell line RT4 showed more distinct bands with [^{3}H]mannose- and [^{3}H]fucose label than the moderately differentiated cell line J82. Cell line T24 derived from a highly malignant bladder carcinoma also had a complex banding pattern with [^{3}H]mannose- and [^{3}H]fucose label, but a very weak expression of [^{3}H]galactose-labelled gps compared to the other bladder carcinoma cell lines. Apart from these general phenotypic differences the individual cell lines could be defined by their marker gps (RT4: completely labelled gps 94, 164, 176 kD; [^{3}H]mannose- and [^{3}H]-fucose-labelled gps at 58, 66, 78, 82, 132 kD; J82: completely labelled gps at 78, 136, 182 kD; [^{3}H]mannose and [^{3}H]fucose-labelled gps at 46, 64, 106, 142 kD; [^{3}H] galactose-labelled gps at 102, 158 kD; T24: completely labelled gps at 134, 182, 200 kD; [^{3}H]galactose- and [^{3}H]mannose-labelled gps at 66, 146 kD; [^{3}H]mannose- and [^{3}H]fucose-labelled gps at 52, 102 kD).

Antigenic changes during long-term *in vitro* culture of these bladder carcinoma cell lines seemed to indicate phenotypic instability [11]. Therefore we analyzed the glycoprotein patterns of the cell lines again after 1 yr of *in vitro* culture in our laboratory (data not shown). By comparison of the patterns, we found that all major glycoprotein markers were still present. We conclude that, at least in this period, the phenotype of the cell remained constant.

Labelling patterns of carcinoma cell lines of various origin and of tumor cell lines of non-epithelial origin

Cervix carcinoma cell line, ME-180, did not express distinct [^{3}H]galactose-labelled gps; however, it did show complex banding patterns with [^{3}H]mannose and [^{3}H]fucose (Fig. 4). Glycoproteins labelled with [^{3}H]fucose were concentrated in the range of 44–58 kD, 84, 94, 103, 112, 134, 154, 186 kD. Some of these gps were also [^{3}H]mannose-labelled. The mamma carcinoma cell line, AlAb, was also characterized by expression of only a few [^{3}H]galactose-labelled gps (85, 92, 104 kD). This cell line had the most bands labelled with [^{3}H]mannose/[^{3}H]fucose (44, 48, 60, 66, 70, 75, 82, 136, 154, 169, 181 kD) and some bands with

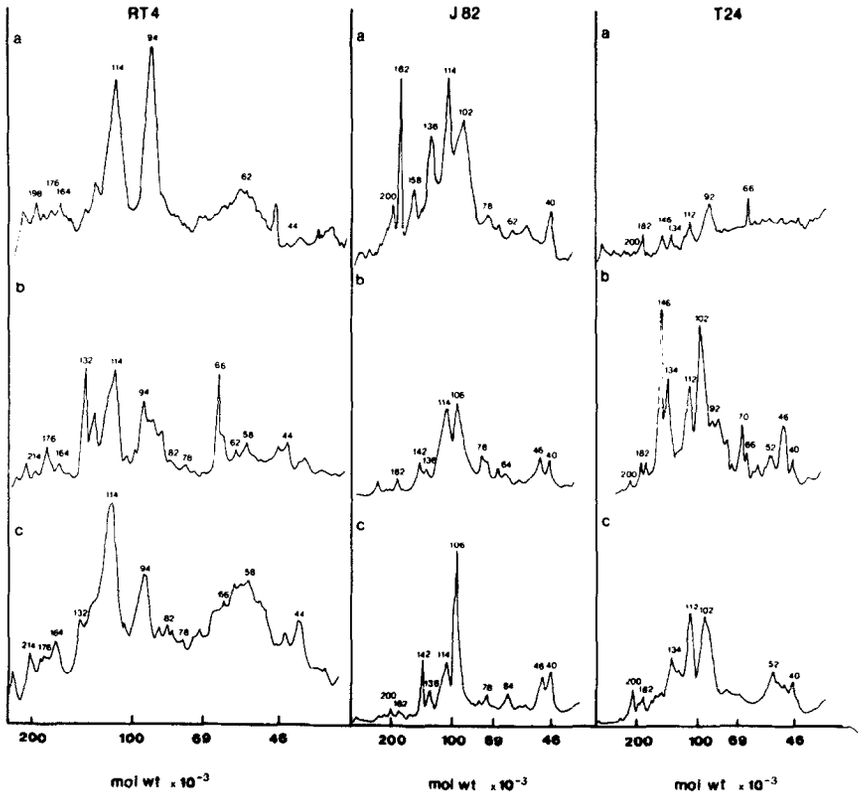

Fig. 3. Scanning profiles of fluorographs of ^{3}H*-labelled glycoproteins derived from human bladder carcinoma cell lines. (a)* [^{3}H]*Galactose label; (b)* [^{3}H]*mannose label; (c)* [^{3}H]*fucose label.*

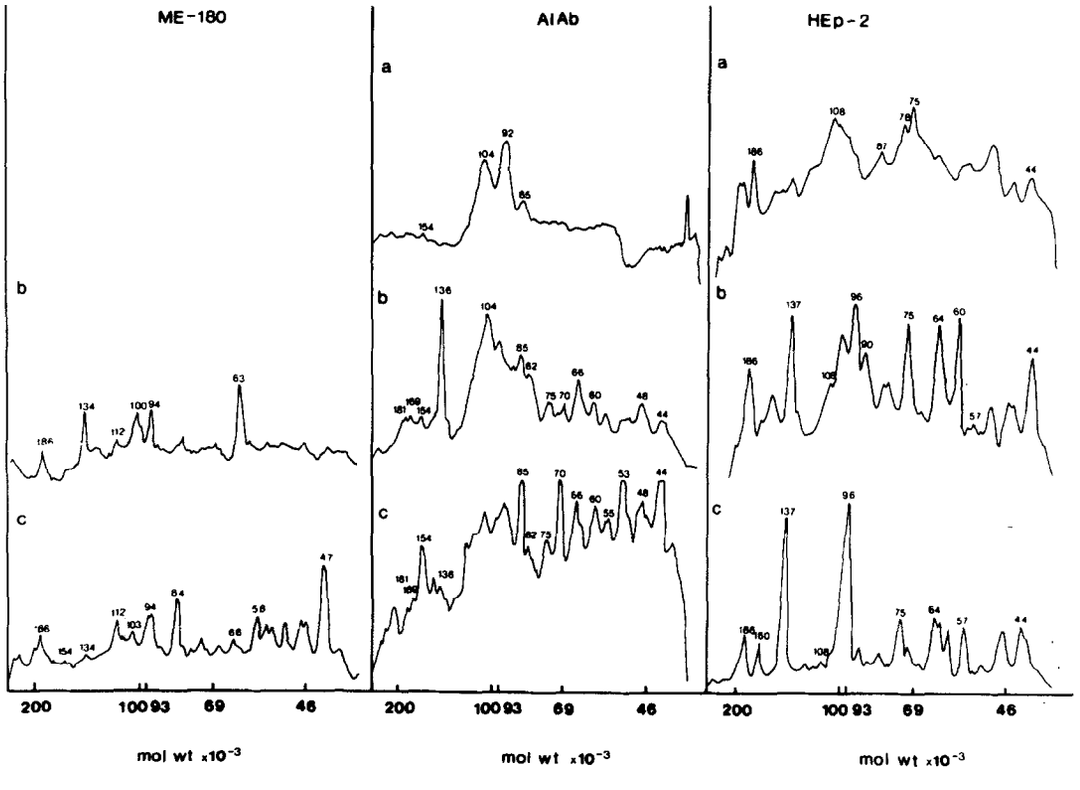

Fig. 4. Scanning profiles of fluorographs of ^{3}H*-labelled glycoproteins derived from various human carcinoma cell lines. (a)* [^{3}H]*Galactose label; (b)* [^{3}H]*mannose label; (c)* [^{3}H]*fucose label.*

[^{3}H]fucose label (53, 55 kD) (Fig. 4). A cell line derived from a metastasis of a larynx carcinoma, HEp-2, had its specific bands with complete label at 75, 108 and 186 kD, two bands with strong [^{3}H]galactose label at 78 and 87 kD and some [^{3}H]mannose/[^{3}H]fucose-labelled bands (57, 96, 137 kD) (Fig. 4).

A melanoma cell line, MML II, was defined by strong expression of completely labelled gps at 82 and 158 kD and by [^{3}H]mannose/[^{3}H]fucose-labelled gps at 62, 64, 96, 132 kD (Fig. 5).

Neuroblastoma cell line, SK-N-SH, showed a very complex banding pattern with all carbohydrate markers which may point to a very differentiated phenotype. By comparing this cell line with the above described cell lines a prospective marker of SK-N-SH could be a completely labelled gp of 190 kD (Fig. 5).

Finally, a sarcoma cell line, P5A, could be distinguished from all tumor cell lines studied by several criteria: (1) [^{3}H]fucose was not incorporated into glycoproteins. This phenomenon has been described for some murine tumor cell lines [19, 20] and may be caused by an enzymatic defect along the fucose-metabolization pathway; (2) [^{3}H]galactose-labelled gps were weakly expressed and only a few distinct gps were seen with [^{3}H]mannose label; (3) glycoproteins of the clusters described being present in the other tumor cell lines, were also absent. This cell line seems to have a very slow turnover of gps and may possess only few functionally relevant surface-bound gps.

DISCUSSION

The carbohydrate moieties of surface gps are located on the external cell surface. A large structural variation of carbohydrate composition in gps provides the necessary selectivity and specificity to serve as receptor molecules. It could be shown, for example, that lymphocytes change the glycosylation pattern of their gps after mitogen or antigen stimulation [21]. Alterations of gps during tumor transformation have been described in numerous reports [22]. The information about glycosylation patterns of human carcinoma cells is still sparse [23].

In this study we used the metabolic labelling method to characterize the glycosylation of gps extracted from a variety of human tumor cell lines. By the use of several carbohydrate precursors labelling different substituents of the oligosaccharide moiety the respective gp patterns of the cell lines could be compared in a very profound manner. Since carbohydrate label [24] and glycoconjugates are enriched on the cell surface [25], we here discuss predominantly differences of surface expressed gps. This easily performed method gives insight not only into gp composition but also into turnover of gps. Besides *de novo* synthesis of gps, it is

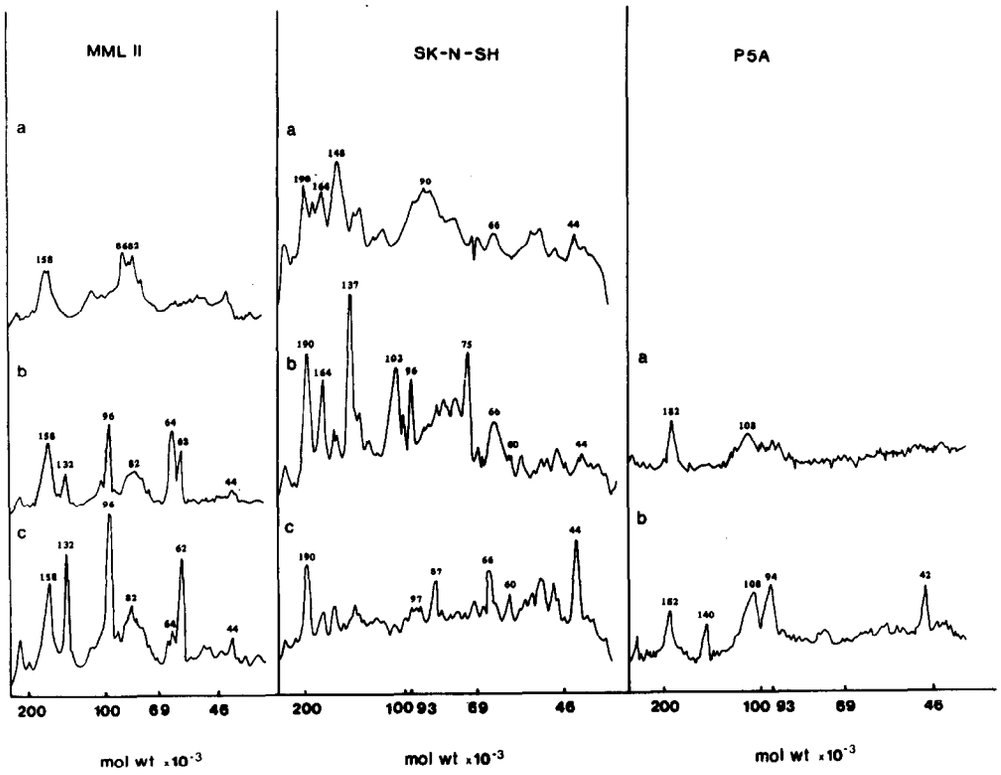

Fig. 5. Scanning profiles of fluorographs of ^{3}H-labelled glycoproteins derived from cell lines MML II (melanoma), SK-N-SH (neuroblastoma) and P5A (sarcoma). (a) [^{3}H]Galactose label; (b) [^{3}H]mannose label; (c) [^{3}H]fucose label.

likely that pre-existing compounds are modified with regard to their glycosylation. This may be demonstrated by gps labelled with [^{3}H]fucose only (e.g. gps of cell line SK-LC-LL, Fig. 1). Since fucose is a terminally situated sugar of gps and is not converted into other sugars [26], we assume that cell lines showing gps only labelled with [^{3}H]fucose either have more defucosylated gps as receptor molecules or have more fucose-binding sites on present fucoproteins compared to other cell lines. Some bands labelled both with [^{3}H]mannose and [^{3}H]fucose may also, at least partly, account for this type of glycoslyation since mannose can be converted into fucose [27]. Furthermore, we observed that glycosylation of gps is not directly dependent on the degree of protein synthesis and proliferation (Table 1).

Patterns of metabolically labelled gps are more complex than those obtained by external labelling via the galactose-oxidase method. Of the cell surface components, only those which carry terminal galactose or galactosamine residues are labelled by the latter method. Frequently occurring gps with terminal fucose or neuraminic acid are therefore difficult to detect. Furthermore, neuraminidase digestion used in NaB^3H_4/galactose oxidase technique may substantially change the molecular weight of the respective glycoproteins. Also, this method does not allow discrimination between gps synthesized by the respective cells and gps attached to the cells. Glycoprotein patterns of the bladder carcinoma cell lines RT4, T24 and J82, the larynx carcinoma cell line HEp2 and the mamma carcinoma cell line ME-180 detected by the external labelling method revealed only a few bands, in contrast to those shown in this paper [4, 28]. Although a direct comparison of external and metabolical labelling methods is difficult because of the above-mentioned reasons, some of these bands seemed to be at the same position as in our study.

In another approach lectins have been used to characterize cell membrane glycoproteins either as ligands in affinity chromatography to purify already extracted glycoproteins [29] or as probes to label surface molecules on intact cells [30]. The efficacy of lectin binding to glycoproteins is influenced above all by the complexity of carbohydrate chains and by the steric accessibility of the sugars recognized. Therefore this method is also limited in its use to gain an overview on total cellular glycoprotein composition.

The metabolic labelling technique allowed each of the cell lines studied to be distinguished by their specific gp patterns. For some cell lines it was shown that these patterns were stable in their expression under standard culture conditions. Similar results were found by Koch *et al.* [31], who compared gps of human pancreatic carcinoma cells at several in vitro culture passages. Therefore, comparison of gp patterns is very useful to monitor cell lines for chromosomal alterations or, frequently occurring, cross-contaminations with other cell lines.

At present we cannot clearly define markers commonly expressed either by human tumors in general or by tumors of the same organ. A gp with similar migration and glycosylation characteristics as gp 175 described above for bladder carcinomas was also identified in ChaGo (176 kD), oat-75 (182 kD) and HT-29 cells (182 kD). It may be that these markers belong to a group of gps which characterize at least some carcinomas. Further investigations with specific monoclonal antibodies could clarify this question. For example, a monoclonal antibody recognizing a 94 kD gp reacted with melanoma cells and also with carcinoma cell lines AlAb, HT-29 and T24 [2]. Glycoproteins of this size were seen in nearly all the carcinoma cells we investigated.

It is possible that gps clustered at molecular ranges of 40–45 kD, 60–62 kD, 80–82 kD and 90–92 kD represent already functionally defined structures such as HLA-class I (46 kD) or the transferrin receptor (90 kD). These molecules have been shown to be expressed in most of the studied cell lines [32]. A sarcoma cell line, P5A, was clearly different in its gp patterns from the other tumor cells. Glycoproteins of 140 and 170 kD described by Lubitz *et al.* [33] for a human sarcoma cell line were also detected by our analysis (140 and 182 kD). These gps may represent markers for sarcoma cells. The heterogeneity of gp expression, apart from some prospective markers, in the carcinoma groups demonstrates how difficult a classification of tumors may be. The characterization of metabolically labelled gps which can be more specified by 2-D gel electrophoresis in combination with the application of specific monoclonal antibodies may help to overcome these difficulties.

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