Differential mucin gene expression in human pancreatic and colon cancer cells

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The purpose of this study was to determine the quantity and nature of the mucins synthesized and secreted by four different pancreatic cancer cell lines. Well- to moderately-differentiated SW1990 and CAPAN-2 human pancreatic cancer cells were found to produce more high- M_r glycoprotein (HMG) than less-differentiated MIA PaCa-2 and PANC-1 cells. Most of the labelled HMG was secreted within 24 h. The results of chemical and enzymic degradation, ion-exchange chromatography and density-gradient centrifugation indicated that the HMG in SW1990 and CAPAN-2 cells has the properties expected for mucins, whereas much of the HMG in MIA PaCa-2 and PANC-1 cells may not be mucin, but proteoglycan. These results are consistent with immunoblots and Northern blots showing the presence of apomucin and apomucin mRNA in SW1990 and CAPAN-2 cells, but not in MIA PaCa-2 and PANC-1 cells. The Western blots and Northern blots also show that SW1990 and CAPAN-2 cells, like breast cancer cells, have the mammary-type apomucin and mRNA coded by the *MUC1* gene, but lack the intestinal type apomucin and mRNA coded by the *MUC2* gene. In contrast, the colon cancer cell lines tested in culture express apomucin and mRNA coded by *MUC2* but not by *MUC1*.

INTRODUCTION

Mucins are high- M_r glycoproteins (HMGs) with a high content of *O*-glycosidically-linked carbohydrate which are produced by several types of epithelial tissues. Many pancreatic-cancer-associated antigens have been found to be present on mucins. These include CA19-9 [1], DU-PAN-2 [2], YPan-1 and SPan-1 [3], all of which are carbohydrate-specific antigens. Little is known about the relative amounts of mucin made by different pancreatic cancer cells or about the types of protein backbone (apomucin) present in pancreatic mucins. The purpose of this study was to determine the quantitative and qualitative differences in the mucins synthesized and secreted by different pancreatic cancer cell lines which show different degrees of differentiation. We investigated four such cell lines, SW1990 [4] and CAPAN-2 [5], which are well- to moderately well-differentiated, and MIA PaCa-2 [6] and PANC-1 [7], which are poorly differentiated. In addition to their different degrees of differentiation, SW1990 [4] and CAPAN-2 [5] cells were chosen because they have been previously shown to produce SPan-1 and YPan-1 cancer-associated mucin antigens [3]. MIA PaCa-2 [6] and PANC-1 cells [7] have been reported to produce high- M_r sulphate-containing glycoconjugates, which have not been well characterized [8,9].

We also sought to determine what type of mucin polypeptide was present in pancreatic cancer cells. Two human mucins have recently been characterized; the mammary mucin encoded by the *MUC1* (also designated *PUM* or *PEM*) gene [10,11], and the intestinal mucin encoded by the *MUC2* (or *SMUC*) gene [12]. Although these two mucins are distinct, both polypeptides have extended regions of tandemly repeated amino acids. The *MUC1*-gene-encoded protein contains 20-amino-acid tandem repeats that contain five threonine and serine residues per repeat unit,

which can serve as sites for *O*-glycosylation. The *SMUC*- or *MUC2*-gene-encoded protein, on the other hand, has a 23-amino-acid repeat, and is much richer in potential *O*-glycosylation sites with 14 threonine residues. The mucin polypeptides (apomucins) can be studied with antibodies against breast cancer apomucin such as 139H2 [13,14], DF3 [15] and HMFG2 [16], antibodies against colon cancer apomucin such as anti-MRP [12] or anti-HFB [17], as well as an antibody against pancreatic cancer apomucin, anti-SWB [18]. Likewise, the cDNA clones for *MUC1* and *MUC2* can be used to examine expression at the level of mRNA by Northern blot analysis.

MATERIALS AND METHODS

Cell lines and cell culture

Human pancreatic adenocarcinoma cell lines SW1990 [4], CAPAN-2 [5], MIA PaCa-2 [6] and PANC-1 [7] were grown in Dulbecco's modified Eagle's medium (DME) containing 10% fetal calf serum (FCS) 100 units of penicillin/ml and 100 µg of streptomycin sulphate/ml. Cultures were maintained at 37 °C in a humidified 7% CO₂ atmosphere.

Metabolic labelling of cells

Cells in 25 cm² culture flasks were labelled with [³H]-glucosamine (10 µCi/ml; specific radioactivity 30 µCi/mmol; ICN Biomedicals, Irvine, CA, U.S.A.) in low-glucose DME/H-16 medium (Grand Island Biological Co., Grand Island, NY, U.S.A.). Cell number was determined from one of triplicate flasks. Total protein and DNA were determined from another of the triplicate flasks, as described [19,20]. After a 24 h labelling period, flasks were chilled on ice, the cell layer was removed using a rubber scraper and the flasks were rinsed with phosphate-

Abbreviations used: HMG, high- M_r glycoprotein; DME, Dulbecco's modified Eagle's medium; FCS, fetal calf serum; PBS, phosphate-buffered saline (0.15 M-NaCl/10 mM-sodium phosphate, pH 7.4); SSC, standard saline citrate (0.15 M-NaCl/15 mM-sodium citrate, pH 7.5).

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buffered saline (PBS). After centrifugation (10 min in a Beckman T-J-6 centrifuge at 170 g), the supernatant was designated as 'medium fraction' and the cell pellet was sonicated on ice in 10 mM-Tris buffer, pH 8.0. The cell homogenate was subjected to ultracentrifugation (100000 g, 1 h) and the supernatant was designated as 'cytosol fraction'.

Gel filtration of labelled glycoproteins

The medium and cytosol fractions were subjected to gel filtration on Sepharose CL-4B (Pharmacia, Uppsala, Sweden) columns with dimensions of 1.27 cm $\times$ 30 cm. Samples of 1 ml were applied to the columns and were eluted with 10 mM-Tris/HCl buffer, pH 8.0. Fractions of 1.2 ml were collected from the column and counted for radioactivity in a scintillation counter.

Chemical and enzymic degradation

[^3H]Glucosamine-labelled HMGs, which were purified from medium fractions of cells labelled with 10 μCi of [^3H]glucosamine/ml, were pooled, dialysed against distilled water and lyophilized. They were analysed after the following treatments. Reductive cleavage of *O*-glycosidic linkages by β -elimination was done in 50 mM-NaOH/1 M-NaBH₄ at 50 °C for 48 h. Trypsin (25 $\mu\text{g}/\text{ml}$) digestion was done at 37 °C for 24 h in 50 mM-Tris/HCl, pH 8.0 [21]. Bovine testicular hyaluronidase was used at a concentration of 5 units/ml at 37 °C for 16 h in 0.1 M-acetate buffer, pH 5.0 [22]. Chondroitinase ABC (EC 4.2.2.4, from *Proteus vulgaris*) was used at a concentration of 5 units/ml in enriched Tris buffer (250 mM-Tris/HCl/176 mM-sodium acetate/250 mM-NaCl, pH 8.0) at 37 °C for 15 h [21]. Heparinase (EC 4.2.2.7, from *Flavobacterium heparinum*) digestion using 5 units/ml was carried out in 0.1 M-Tris/HCl, pH 7.2, at 37 °C for 24 h [23]. Pronase E (1 % final concentration, preincubated for 30 min at 50 °C to inactivate glycosidases) was used at 50 °C for 24 h in PBS containing 0.02 M-CaCl₂ [24]. All of these enzymes were obtained from Sigma (St. Louis, MO, U.S.A.). Enzyme units are based on the supplier's specification. The treated samples were re-chromatographed on Sepharose CL-4B columns (1.27 cm $\times$ 30 cm) in 10 mM-Tris/HCl buffer, pH 8.0. Fractions of 1.2 ml were collected from the column and counted for radioactivity in a scintillation counter.

DEAE-cellulose chromatography

Lyophilized void volumes of medium fractions (5×10^3 – 20×10^3 c.p.m.) from [^3H]glucosamine-labelled materials were dissolved in 10 mM-Tris, pH 8.0, and applied to a DEAE-cellulose column (1 cm $\times$ 2 cm) according to Podolsky & Isselbacher [25]. The column was eluted with a discontinuous NaCl gradient (0–300 mM-NaCl in 50 mM increments) in 10 mM-Tris, pH 8.0, followed by 1 % acetic acid. Fractions of 3 ml were collected and 1 ml of each fraction was counted for radioactivity.

CsCl gradient ultracentrifugation

To the [^3H]glucosamine-labelled void volume fractions collected from media of the four cell lines, CsCl (0.54 g/ml) was added, and the CsCl solution was centrifuged for 72 h at 1.6×10^5 g in a Beckman SW-41 rotor at 5 °C. Fractions of 1.0 ml were removed, weighed to determine density and counted for ^3H .

Glycosyltransferase activity

The glycosyltransferase assays used in this study were modifications of earlier methods [26]. The assays using triplicate samples were repeated three times in each cell line. The assay mixtures for

polypeptidyl *N*-acetylgalactosaminyltransferase and β 1,3-galactosyltransferase contained 0.1 M-cacodylate buffer, pH 6.8, 20 mM-MnCl₂, 0.1 % Triton X-100, 200 μM -ATP and 25–100 μg of enzyme in a total volume of 0.1 ml. The acceptor for polypeptidyl *N*-acetylgalactosaminyltransferase was apo bovine submaxillary mucin (100 μg), and the donor was 5.4 μM -UDP- ^3H -acetylgalactosamine (4×10^4 c.p.m.). For β 1,3-galactosyltransferase, asialo-ovine submaxillary mucin (100 μg) and 0.12 μM -UDP- ^3H -galactose (25×10^4 c.p.m.) were used as acceptor and donor respectively.

The reaction mixtures were incubated at 37 °C for 90 min and were then precipitated with 1 % phosphotungstic acid in 0.5 M-HCl. Net radioactivity transferred to the exogenously added acceptors was determined after subtracting the endogenous activity.

SDS/PAGE and Western blots

Electrophoresis was performed according to the method of Laemmli [27], with a 7 % polyacrylamide running gel and a 4 % stacking gel. Samples (homogenates of the four pancreatic cancer cell lines, and of two colon cancer cell lines, HM7 [28] and H498 [29], as well as two breast cancer cell lines, MCF7 [30] and ZR-75-1 [31]) were heated at 100 °C in 2.5 % mercaptoethanol and 1.2 % SDS for 5 min prior to electrophoresis.

For immunoblot analysis, proteins were transferred electrophoretically overnight to nitrocellulose [32], soaked for 1 h at room temperature in 0.5 % (w/v) Carnation non-fat dry milk in PBS, and soaked for 1 h at room temperature in blocking buffer (2 % BSA in 10 mM-Tris/0.15 M-NaCl/0.1 % NaN₃, pH 7.4), and rinsed in PBS. The nitrocellulose membranes were incubated with rabbit antibodies (IgG fractions of anti-SWB [18], anti-HFB [17] and anti-MRP [12], purified on a Protein A-Sepharose column and diluted 1:50 in the blocking buffer) for 1 h, then with ^{125}I -labelled Protein A (10^6 c.p.m./ml) in the blocking buffer for 1 h [33]. Alternatively, the membranes were incubated with mouse monoclonal antibodies for 1 h, and then with rabbit anti-mouse IgG (Zymed Laboratories, South San Francisco, CA, U.S.A.) diluted 1:400 in the blocking buffer for 1.5 h, followed by ^{125}I -labelled Protein A (10^6 c.p.m./ml) in the blocking buffer for 1 h [33]. The mouse monoclonal antibodies were kindly donated by J. Hilken (139H2 [13,14], ascites fluid diluted 1:200 in the blocking buffer), D. Kufe (DF3 [15], ascites fluid diluted 1:1000 in the blocking buffer) and J. Taylor-Papadimitriou (HMFG2 [16], culture medium diluted 1:10 in the blocking buffer). The sheet was washed between incubations with PBS containing 0.05 % Nonidet P-40. X-Omat AR2 film (Kodak) was exposed to the nitrocellulose with an intensifying screen (Cronex Lighting Plus, DuPont) at –70 °C.

Proteins were visualized by staining the gels with 0.25 % Coomassie Brilliant Blue R-250. The following proteins were used as molecular mass standards: myosin, β -galactosidase, phosphorylase B, BSA, ovalbumin and carbonic anhydrase (Sigma). Normal rabbit serum or myeloma (P3-X63-Ag 8.653) ascitic fluid was used for the control experiments for rabbit antibodies or mouse monoclonal antibodies respectively.

Northern blot analysis

RNA was extracted from each cell line using 6 M-urea and 3 M-LiCl [34]. For Northern blot analysis, 30 μg of the total RNA for each sample was subjected to electrophoresis in 1.5 % agarose gels containing 6.6 % formaldehyde. A buffer system consisting of 10 mM-sodium phosphate (pH 7.0), 1 mM-EDTA and 5 mM-sodium acetate was used for electrophoresis. Following capillary transfer of the RNA to Biotrans A nylon membranes using 20 $\times$ SSC and baking at 80 °C in a vacuum oven, prehybridization

was conducted at 42 °C for 18–22 h in 5 × Denhardt's solution/5 × SSC/50 mM-sodium phosphate (pH 6.5)/0.1 % SDS salmon sperm DNA (250 µg/ml)/50 % deionized formamide [35]. Labelling of *MUC1* cDNA, *MUC2* cDNA [12] and glyceraldehyde-3-phosphate dehydrogenase probes [36] was done by the random hexamer primer reaction [37], and hybridization was conducted as for prehybridization, except that $(1-6) \times 10^5$ c.p.m. of ^{32}P -labelled probe/ml and 10 % dextran sulphate were used. The membranes were then washed with 2 × SSC containing 0.1 % SDS for 1.5 h at room temperature, followed by 1 h washes at room temperature and then 1 h at 55 °C in 0.1 × SSC/0.1 % SDS. After these washings, the membranes were autoradiographed. After autoradiography, blots were erased, checked for residual radioactivity and rehybridized with subsequent probes.

The *MUC1* cDNA clone, PUM24P, was isolated from a λ gt11 cDNA library prepared from the cell line MCF7, kindly provided by P. Chambon (University of Strasbourg). The first screening was done using the monoclonal antibody LICR.LON M8 [38], kindly provided by the Ludwig Institute for Cancer Research (London, U.K.). This antibody recognizes the *MUC1* gene products on immunoblotting [39,40], and there was prior evidence that it recognized a protein epitope [38]. Antibody-positive recombinants were further screened using an oligonucleotide (30-mer) synthesized from the published repeat sequence [41,42]. One positive clone, PUM24P, was subcloned into M13 and PUC13, fully sequenced and found to comprise 4.6 repeat units of the *MUC1* cDNA.

RESULTS

HMG glycoprotein synthesis

The synthesis of HMG in the cell layer and its secretion into the medium were estimated by measuring the amount of [^3H]-glucosamine-labelled glycoproteins excluded on Sepharose CL-4B. The radioactivities of the void volume for each cell line were compared on the basis of cell number and protein amount (Table 1). SW1990 and CAPAN-2 cells had about 3.5–8-fold more [^3H]-glucosamine-labelled HMG secreted into the medium than did MIA PaCa-2 and PANC-1 cells. When HMG synthesis was normalized on the basis of DNA amount (results not shown), SW1990 and CAPAN-2 cells still had more than MIA PaCa-2 and PANC-1 cells. Less pronounced differences were observed when the HMGs in the cytosol fractions were compared.

Chemical and enzymic degradation of HMG

The labelled HMG from the medium of SW1990 and CAPAN-2 cells was predominantly mucin, whereas that from MIA PaCa-2 and PANC-1 cells was susceptible to treatments that degrade

proteoglycans (Table 2). Thus 69 % of the HMG of SW1990 cells and 90 % of the HMG of CAPAN-2 cells was not degraded by the hyaluronidase treatment, whereas 73 % of the HMG of MIA PaCa-2 cells and 91 % of the HMG of PANC-1 cells were degraded. Furthermore, 79 % of the HMG of SW1990 cells and 92 % of the HMG of CAPAN-2 cells were not degraded by the chondroitinase ABC treatment, whereas 64 % of the HMG of MIA PaCa-2 cells and 79 % of the HMG of PANC-1 cells were degraded. By correcting for the extent of degradation by hyaluronidase, we estimated the amounts of labelled mucin synthesized by each cell line (Table 3). We conclude that SW1990 and CAPAN-2 cells secreted over 15-fold more mucin than MIA PaCa-2 and PANC-1 cells.

DEAE-cellulose chromatography

The [^3H]-glucosamine-labelled HMGs purified by gel filtration of SW1990 and CAPAN-2 cell medium had peak III (eluted by 150 mM-NaCl) as their major component (Figs. 1a and 1b). The HMGs from the medium of MIA PaCa-2 and PANC-1 cells, however, had peak V (eluted by 250 mM-NaCl) as their major component (Figs. 1c and 1d). When unlabelled SW1990 xenograft mucin [18], detected by binding of peroxidase-labelled *Ricinus communis* agglutinin, was chromatographed in the same fashion, peak III was also the major species (results not shown). These data indicate that the HMG of MIA PaCa-2 and PANC-1 cells is more acidic than that of SW1990 and CAPAN-2 cells, consistent with the conclusion that the HMG in SW1990 and CAPAN-2 cells is mucin, while much of the HMG in MIA PaCa-2 PANC-1 cells is proteoglycan.

CsCl gradient ultracentrifugation

The HMGs purified from the medium by gel filtration on Sepharose CL-4B were further examined on density gradients. In SW1990 and CAPAN-2 cells, the peak of ^3H was found at a density of 1.405 g/ml and 1.432 g/ml respectively (Figs. 2a and 2b). However, in MIA PaCa-2 and PANC-1 cells, the peak of ^3H

Table 1. Radioactivity of [^3H]-glucosamine-labelled glycoproteins eluted in the void volume of a Sepharose CL-4B column

The results are means ($\pm$ S.E.M.). M, medium; C, cytosol.

Cell line	^3H radioactivity in glycoproteins			
	(d.p.m./ 10^3 cells)		(d.p.m. µg of protein)	
	M	C	M	C
SW1990	1022 $\pm$ 37	502 $\pm$ 168	3184	1564
CAPAN-2	1008 $\pm$ 107	193 $\pm$ 31	3892	745
MIA PaCa-2	129 $\pm$ 48	106 $\pm$ 34	533	438
PANC-1	248 $\pm$ 89	132 $\pm$ 12	915	487

Table 2. Degradation of [^3H]-glucosamine-labelled medium HMG

Treatment	Cell line...	Void volume ^3H (% of total)			
		SW1990	CAPAN-2	MIA PaCa-2	PANC-1
Control		100	100	100	100
Trypsin		66	65	69	68
Pronase		2	3	0	0
NaOH/NaBH ₄		0	0	0	0
Hyaluronidase		69	90	27	9
Chondroitinase ABC		79	92	36	21
Heparinase		77	93	59	82

Table 3. Estimated amounts of secreted mucin, corrected for hyaluronidase-susceptible ^3H

Cell line	Secreted [^3H]mucin	
	(d.p.m./ 10^3 cells)	(d.p.m. µg of protein)
SW1990	700	2181
CAPAN-2	902	3483
MIA PaCa-2	35	146
PANC-1	22	80

Fig. 1. DEAE-cellulose chromatography of [^3H]glucosamine-labelled HMG from media of pancreatic cell lines SW1990 (a), CAPAN-2 (b), MIA PaCa-2 (c) and PANC-1 (d).

Void volumes of medium fractions from [^3H]glucosamine-labelled materials were chromatographed on DEAE-cellulose as described in the Materials and methods section. Arrows, left to right, show elution with 50, 100, 150, 200, 250 and 300 mM-NaCl.

was found at a density around 1.50 g/ml (Figs. 2c and 2d). This result is also consistent with the conclusion that the majority of the HMG of SW1990 and CAPAN-2 cells is mucin, but that the majority of the HMG of MIA PaCa-2 and PANC-1 cells is not mucin, but proteoglycan.

Glycosyltransferase activity

CAPAN-2 and, to a lesser extent, SW1990 cells had higher levels of polypeptidyl *N*-acetylgalactosaminyltransferase and β 1,3-galactosyltransferase than did MIA PaCa-2 and PANC-1 cells (Table 4). However, the difference in enzyme activity was not as great as the differences in the extent of [^3H]glucosamine incorporation (Table 1).

Comparison of apomucin among pancreatic, colonic and breast cancer cell lines

The nature of mucin polypeptides was studied in the cell homogenates of pancreatic (SW1990, CAPAN-2, MIA PaCa-2, PANC-1), colonic (HM7, H498) and breast (MCF7, ZR-75-1) cancer cells by immunoblot analysis. When the immunoblots were incubated with monoclonal antibody against the breast cancer apomucin, 139H2 (Fig. 3a), there were two discrete bands of apparent M_r 165000 and 230000 in SW1990 and CAPAN-2 cells. The other two types of pancreatic cancer cells, MIA PaCa-2 and PANC-1, showed no bands reactive with the 139H2 antibody. Of the two breast cancer cells, MCF7 showed three discrete bands of apparent M_r 165000, 220000 and 260000, and ZR-75-1 had four discrete bands of apparent M_r 165000, 180000, 220000 and 270000. The bands detected in MCF7 cells were weaker than those of the other cell lines. The two colonic cancer cell lines showed no 139H2-reactive bands. Antibodies DF3 and HMFG2 (results not shown) gave similar results.

When the immunoblots were incubated with antibody against a synthetic mucin repeat peptide of colon cancer apomucin, anti-MRP (Fig. 3b), HM7 cells showed an intense band at the top of the 7% acrylamide separating gel, though there were weak bands of apparent M_r 100000 and 165000, and a weak broad band of

Fig. 2. CsCl gradient ultracentrifugation profiles of [^3H]glucosamine-labelled HMG from media of pancreatic cell lines SW1990 (a), CAPAN-2 (b), MIA PaCa-2 (c) and PANC-1 (d).

The broken line indicates density.

Table 4. Glycosyltransferase activities of cell lines

The results are means $\pm$ S.E.M. for three separate homogenates.

Cell line	Enzyme activity (pmol/h per mg)	
	Polypeptidyl GalNAc transferase	β 1,3-Gal transferase
SW1990	589 $\pm$ 179	15.2 $\pm$ 2.7
CAPAN-2	1042 $\pm$ 196	32.5 $\pm$ 10.6
MIA PaCa-2	367 $\pm$ 168	7.5 $\pm$ 1.5
PANC-1	433 $\pm$ 53	9.1 $\pm$ 2.4

about apparent M_r 220000. Another colon cancer cell line, H498, showed a pattern similar to that of HM7, but the intensity of the bands was weaker. The pancreatic cancer cells and breast cancer cells showed no antigen reactive with anti-MRP. Anti-HFB, an antibody prepared against deglycosylated colon cancer mucin [17] gave similar results (not shown).

When immunoblots were incubated with antibody against pancreatic cancer apomucin, anti-SWB (Fig. 3c), SW1990 and CAPAN-2 cells showed two discrete bands of apparent M_r 165000 and 230000 that coincided with those detected by 139H2. PANC-1 cells showed a faint band of apparent M_r 165000, but MIA PaCa-2 cells were negative. The two discrete bands of apparent M_r 165000 and 220000 faintly detected in MCF7 cells, and the three bands of apparent M_r 165000, 180000 and 220000 in ZR-75-1 cells, also coincided with those in the immunoblots incubated with the anti-MUC1 antibody 139H2, but the band of apparent M_r 260000 in MCF7 cells and the band of apparent M_r 270000 in ZR-75-1 cells were not detected with anti-SWB. One of the colon cancer cell lines, HM7, showed intense positive staining at the top of the separating gel and also showed bands of apparent M_r 165000 and 220000. Another colon cancer cell line, H498, showed a weak band of apparent M_r 165000.

Fig. 3. Immunoblots of HMG from various cell lines

Portions of cellular protein (75 µg/lane) from two breast cancer cell lines (MCF7, ZR-75-1), two colon cancer cell lines (HM7, H498) and four pancreatic cancer cell lines (SW1990, CAPAN-2, MIA PaCa-2, PANC-1) were subjected to electrophoresis, transferred to nitrocellulose, and immunoblotted with 139H2 (a), anti-MRP (b) or anti-SWB (c) antibodies as described in the Materials and methods section. The arrow shows the interface between stacking and separating gels. The arrowheads show the migration positions of M_r markers.

Fig. 4. Northern blots of RNA from various cell lines

Total cellular RNA (30 µg) from two breast cancer cell lines (MCF7, ZR-75-1), two colon cancer cell lines (HM7, H498) and four pancreatic cancer cell lines (SW1990, CAPAN-2, MIA PaCa-2, PANC-1) was subjected to electrophoresis, transferred to nylon membranes, and hybridized with *MUC1* cDNA (a) and *MUC2* cDNA (b) probes, as described in the Materials and methods section.

DISCUSSION

The metabolic labelling of the cell lines using [3 H]glucosamine revealed that SW1990 and CAPAN-2 cells make more HMG than do MIA PaCa-2 and PANC-1 cells, and that a large proportion of this HMG is secreted within 24 h. To determine whether the labelled HMG is indeed mucin, and also to quantify the amount of mucin made, we carried out experiments of chemical and enzymic degradation, ion-exchange chromatography and density-gradient centrifugation of Sepharose-CL-4B-excluded material. The results demonstrated that the HMG from SW1990 and CAPAN-2 cells has the properties expected for mucins. In contrast, much of the HMG in MIA PaCa-2 and PANC-1 cells appears not to be mucin, but proteoglycan, since it is largely degraded by hyaluronidase and chondroitinase. By correcting for the proportion of HMG that is sensitive to hyaluronidase digestion, we can estimate that SW1990 and CAPAN-2 cells secrete at least 15-fold more labelled mucin than do MIA PaCa-2 and PANC-1 cells. CAPAN-2 and SW1990 cells also have somewhat higher activities of two of the glycosyltransferases responsible for mucin glycosylation than do the other cell lines. The amount of mucin produced, however, as determined by glucosamine incorporation, is a direct reflection of the level of expression of the apomucin, as assessed by immunoblots and Northern blots.

Previous studies indicate that human pancreatic ductal epithelium synthesizes and secretes mucin [43]. Most pancreatic carcinomas are believed to arise from the ductal epithelium [44]. The SW1990 cell line was established from a Grade II adenocarcinoma, forms xenograft tumours similar to the original neoplasm in nude mice, and has characteristics of ductal epithelium [4]. Although the extent of differentiation of the original tumours from which the CAPAN-2 cell line was established is not known [5], it forms a well-differentiated ductal cell adenocarcinoma in nude mouse xenograft (Y. S. Kim, unpublished work). The MIA PaCa-2 cell line was established from an undifferentiated carcinoma [6] and has less ductal-like characteristics [8]. This difference between these cell lines seems to be correlated with the difference in mucin production between them. The PANC-1 cell line, which was established from an undifferentiated carcinoma and forms anaplastic carcinoma in nude mice [7], has maintained some of the differentiated characteristics of normal pancreatic ductal epithelial cells [9]. PANC-1 cells secrete more labelled HMG than do MIA PaCa-2 cells, but most of it seems to be not a mucin but a proteoglycan. Madden *et al.* [8,9] found that MIA PaCa-2 and PANC-1 cells synthesize

Northern blot analysis

MUC1 mRNA was detected in SW1990 and CAPAN-2 cells and in the breast cell lines as expected [10,11], although the message level in MCF7 was somewhat lower than the others (Fig. 4a). The other two pancreatic cancer cell lines and the two colon cancer cell lines showed little or no apomucin mRNA detected by *MUC1* cDNA.

Only the two colon cancer cell lines, HM7 and H498, showed apomucin mRNA detectable by *MUC2* cDNA, which codes for colon cancer-type apomucin [12] (Fig. 4b). Both *MUC1* and *MUC2* mRNA seem to be inherently polydisperse, since a control probe for glyceraldehyde-3-phosphate dehydrogenase gave a single discrete band.

These findings were consistent with the results of the Western blots showing apomucin detected by 139H2 cells in the breast and pancreatic cancer cell lines but not the colonic cell lines tested, and apomucin detected by anti-MRP in colonic but not in breast or pancreatic cell lines.

and secrete sulphated macromolecules into the medium, which were suggested to be sulphomucins. Sulphated macromolecules may be either sulphated mucins or proteoglycans. However, our data indicate that the MIA PaCa-2 and PANC-1 cell lines synthesize and secrete predominantly proteoglycan.

From the results of immunoblots and Northern blots, we conclude that the two well- to moderately-differentiated pancreatic cancer cell lines, SW1990 and CAPAN-2, like the two breast cancer cell lines, express the *MUC1* gene but not the *MUC2* gene. The differences between cell lines in the electrophoretic mobility of the immunoreactive *MUC1* proteins (Fig. 3) are consistent with the polymorphism, due to a variable number of tandem repeats, in the *MUC1* gene [10,11].

Anti-SWB, which is an antibody against completely deglycosylated SW1990 xenograft mucin [18], detected bands that are similar in electrophoretic mobilities to those stained by 139H2, DF3 and HMFG2 monoclonal antibodies. Thus anti-SWB seems to react with *MUC1* gene products. However, anti-SWB also reacted with colon cancer cells, which express the *MUC2* gene but not the *MUC1* gene. Since anti-SWB, like anti-MRP, reacted with colon cancer cell products mainly at the top of the separating gel, anti-SWB seems also to react with *MUC2* gene products. This suggests that SW1990 xenografts, unlike SW1990 cells in culture, express not only breast cancer cell type mucin (*MUC1* gene product), but also colon cancer cell type mucin (*MUC2* gene product). This difference in expression may reflect a situation similar to that observed previously for the LS174T colon cancer cell line, which expresses much more mucin in xenografts than in cell culture [45].

Recently, Lan *et al.* [46,47] reported that pancreatic apomucin and breast apomucin share some similarity in tandem-repeated nucleic acid and protein sequences. Cloning and sequencing of cDNAs from the HPAF pancreatic cancer cell line [47] confirmed the presence of *MUC1* mucin almost identical to that in breast cancer cell lines [48,49]. Our results in pancreatic and breast cancer cell lines are consistent with their results. In addition, we also observed that both pancreatic and breast cancer cells lack colon-cancer-type apomucin. Thus there seems to be an obvious difference in the apomucin present in pancreatic and breast cancer cells in comparison with that in colon cancer cells.

Further studies will be necessary to resolve the questions of whether pancreatic cancer cells have other distinct types of mucins, and how regulation of the type of mucin expressed differs in cultured cells and in xenografts.

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