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Characterization of a human cervical carcinoma-associated antigen by lectin binding and immuno-electron microscopy

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Summary. The specific binding and nature of the epitope recognized by monoclonal antibody (Mab) 1H10, which binds an antigen expressed on human cervical tumors, was characterized by enzyme digestion, lectin competition assay and immuno-electron microscopy. Membrane homogenates of CaSki cervical carcinoma cells were digested with various enzymes, then analysed by SDS-PAGE and immunoblotting. Cells grown on coverslips were treated with various enzymes and in situ binding of Mab 1H10 to cells was analysed by electron microscopy. The ability of lectin-conjugates to block Mab 1H10 binding to CaSki cells was also examined. Treatment of samples with sodium periodate abrogated antigen recognition by Mab 1H10. Neuraminidase and hyaluronidase digestion decreased but did not eliminate Mab 1H10 binding to cells in situ. Chondroitinase ABC digestion, in contrast, removed Mab 1H10 binding sites both in vitro and in situ. Trypsin and chymotrypsin digestion of cell membrane homogenates decreased the molecular weight of the Mab 1H10 antigen but did not decrease the binding intensity. Wheat germ agglutinin (WGA) strongly bound to CaSki cells and partially blocked Mab 1H10 binding, indicating that the antigen contains *N*-acetyl-galactosamine residues at or near the epitope recognized by Mab 1H10. *Ricinus communis* agglutinin (RCA) exhibited a similar binding pattern to WGA. However, concanavalin A bound only weakly to CaSki cells and was ineffective at blocking Mab 1H10 binding. The tumor-associated antigen recognized by Mab 1H10 is concluded to be a chondroitin sulphate glycoprotein or proteoglycan rather than a mucopolysaccharide or lipoprotein.

Introduction

A monoclonal antibody, Mab 1H10, was recently produced which recognizes a surface antigen expressed on CaSki cervical carcinoma cells and several other tumor cell lines (Roffler et al. 1991a; Yeh et al. 1992). Mab 1H10 binds to 40% of tumor specimens of human cervical carcinoma as well as about 36% of colorectal carcinoma tissues and some ovarian, bladder and lung tumor tissues (Table 1). Mab 1H10 has not been found to bind normal human cells and tissues. This antibody is of clinical interest due to its selective reaction with human cervical and other carcinoma tissues (Roffler et al. 1991a). Cervical carcinoma is a common lethal malignancy affecting women, especially African-Americans (Swanson

Table 1. Immunohistochemical reaction of Mab 1H10 with human cells and tissues[a]

Carcinoma	Positive/ tested	Normal	Positive tested
Cervical carcinoma	40/97	Cervix	0/24
Ovarian carcinoma	1/3	Ovary	0/1
Endometrial carcinoma	0/2	Endometrium	0/2
Breast carcinoma	0/7	Breast	0/4
Hepatoma	0/6	Liver	0/14
Colorectal carcinoma	8/22	Colon	0/4
Bladder carcinoma	2/12	Bladder	0/2
Renal cell carcinoma	0/1	Kidney	0/2
Lung carcinoma	1/5	Lung	0/3
Prostate carcinoma	0/4	Urethra	0/1
Testicular carcinoma	0/5	Thyroid	0/2
Stomach carcinoma	1/1[b]	Oesophagus	0/1
Osteogenic sarcoma	1/1	Cerebrum	0/3
Glioma	0/3	Spleen	0/8
Meningioma	0/3	Lymphocytes	0/5
Astrocytoma	0/2	RBC (A)	0/5
		RBC (B)	0/4
		RBC (O)	0/4

[a] Reaction of Mab 1H10 with 5 µM cryostat tissue sections was determined by the peroxidase anti-peroxidase technique. Positive reaction was determined by comparison with control IgG_3 background staining. Antibody binding to red blood cells (RBC) and lymphocytes was examined by haemagglutination and immunofluorescence assays respectively
[b] Weak reaction

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et al. 1983) and Asians (Yeh 1985). Despite the widespread use of programmes of cervical cytological screening, cervical carcinoma is still a serious problem (Murphy et al. 1988; Dodgson et al. 1989). Monoclonal antibodies against cervical carcinoma may yield clinical benefits through improved screening, staging and treatment. Solid subcutaneous cervical carcinoma tumors in nude mice were effectively suppressed by intraperitoneal cavity or intravenous administration of Mab 1H10 conjugated with *Pseudomonas* exotoxin A (Roffler et al. 1991b) or doxorubicin (Yeh et al. 1992). Our previous work suggested that Mab 1H10 binds to a glycoconjugate present on the surface of cells of the cervical carcinoma cell line CaSki (Roffler et al. 1990a). However, the biochemical nature of this antigen is still unknown.

Characterization of glycoprotein antigens is difficult due to the complexity of carbohydrates. The effect on antibody binding of removing terminal monosaccharide residues from mucopolysaccharide is a useful technique for studying tumor antigens (Plendl and Schumacher 1989; Pancino et al. 1991). Enzymes that remove specific terminal monosaccharides, such as neuraminidase which removes sialic acid, and hyaluronidase which removes hyaluronic acid, have been used to determine which terminal monosaccharide residues are important for antigen-antibody interactions (Bischof and Lodish 1987; Wang 1989; Wang et al. 1990). Lectin molecules which bind to specific monosaccharides are also useful for studying antibody binding to carbohydrates. Lectins have been used to study cell agglutination and mitogenicity (Goldstein et al. 1980) as well as to investigate the oligosaccharides involved in cell-cell interactions, cell-virus interactions and the epitopes on surface antigens (Sheer et al. 1988; Taatjes et al. 1990; Wang et al. 1990). In this study, *Triticum vulgaris* agglutinin (WGA), *Ricinus communis* agglutinin (RCA) and *Concanavalin ensiformis* (Con A) were examined for their ability to block Mab 1H10 binding to CaSki cells. Three methods have been applied, including enzyme digestion, lectin cytochemistry and immuno-electron microscopy, to characterize a human cervical carcinoma-associated antigen identified by Mab 1H10.

Materials and methods

Cells

CaSki cells were maintained in RPMI 1640 culture medium (Gibco, Grand Island, NY, USA) supplemented with 5% heat-inactivated fetal calf serum, 100 U/ml penicillin and 100 µg/ml streptomycin. Cells were tested routinely for mycoplasma contamination by a standard method (Russell et al. 1975).

Production of monoclonal antibody 1H10

Mab 1H10 was developed by the hybridoma technique (Köhler and Milstein 1975) as previously described (Roffler et al. 1991a). Mab 1H10, a murine IgG_3 monoclonal antibody, was purified from ascites fluid produced in BALB/c mice or from the supernatant of culture medium obtained from hybridomas grown in serum-free medium using an Opticell 5200R cell culture system. Mab activity was determined by enzyme-linked immunosorbent assay (Roffler et al. 1991a).

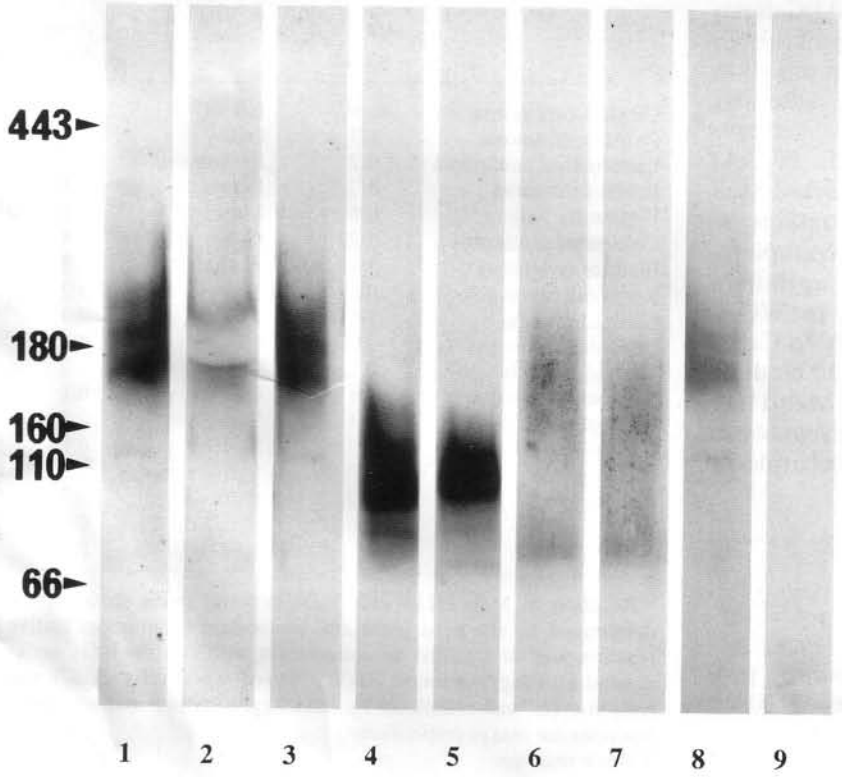

Fig. 1. Immunoreactivity of Mab 1H10 antigen after enzymatic digestion. Membrane homogenates isolated from CaSki cells were digested with enzymes (see the Materials and methods), then analysed by SDS-PAGE and immunoblotting with Mab 1H10. *Lane 1*, untreated membrane fraction; lanes 2–8, digestion with: *lane 2*, chondroitinase ABC; *lane 3*, neuraminidase; *lane 4*, trypsin; *lane 5*, chymotrypsin; *lane 6*, papain; *lane 7*, protease; *lane 8*, lipase; *lane 9*, sodium periodate treatment. Membrane components were not fixed with glutaraldehyde before enzyme treatment

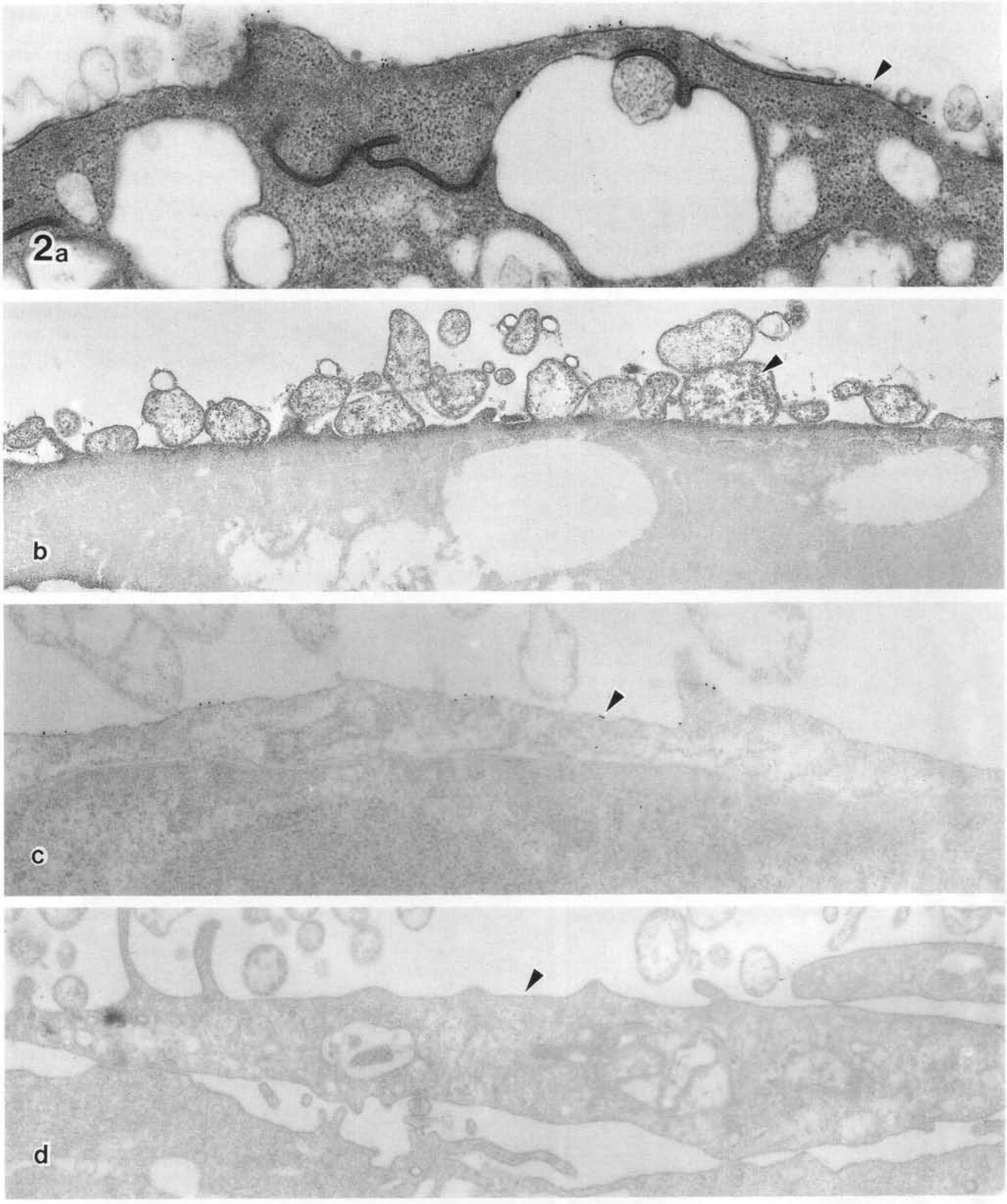

Fig. 2a–d. Electron micrographs of binding of Mab 1H10 to CaSki cells in situ. **a** Unfixed CaSki cells in suspension were immunolabelled with Mab 1H10 and Ab-gold (10 nm). Clusters of gold particles appear dispersed on the cell surface (*arrowhead*). **b** CaSki cells grown on a plastic coverslip were fixed and incubated with Mab 1H10 and Ab-PO. Positive reactions were seen on the apical surfaces and in vesicles (*arrowhead*) attached on the surface. **c** Fixed CaSki cells incubated with Mab 1H10 and Ab-gold. Gold particles are evident on the cell surface (*arrowhead*) but not on vesicles outside the membrane, similar to the pattern in **a**. **d** No peroxidase reaction was seen on the surface (*arrowhead*) when Mab 1H10 was omitted before incubation of fixed CaSki cells with Ab-peroxidase (PO) complex. Magnification: **a** ×32400; **b** ×13700; **c** ×27300; **d** ×16500

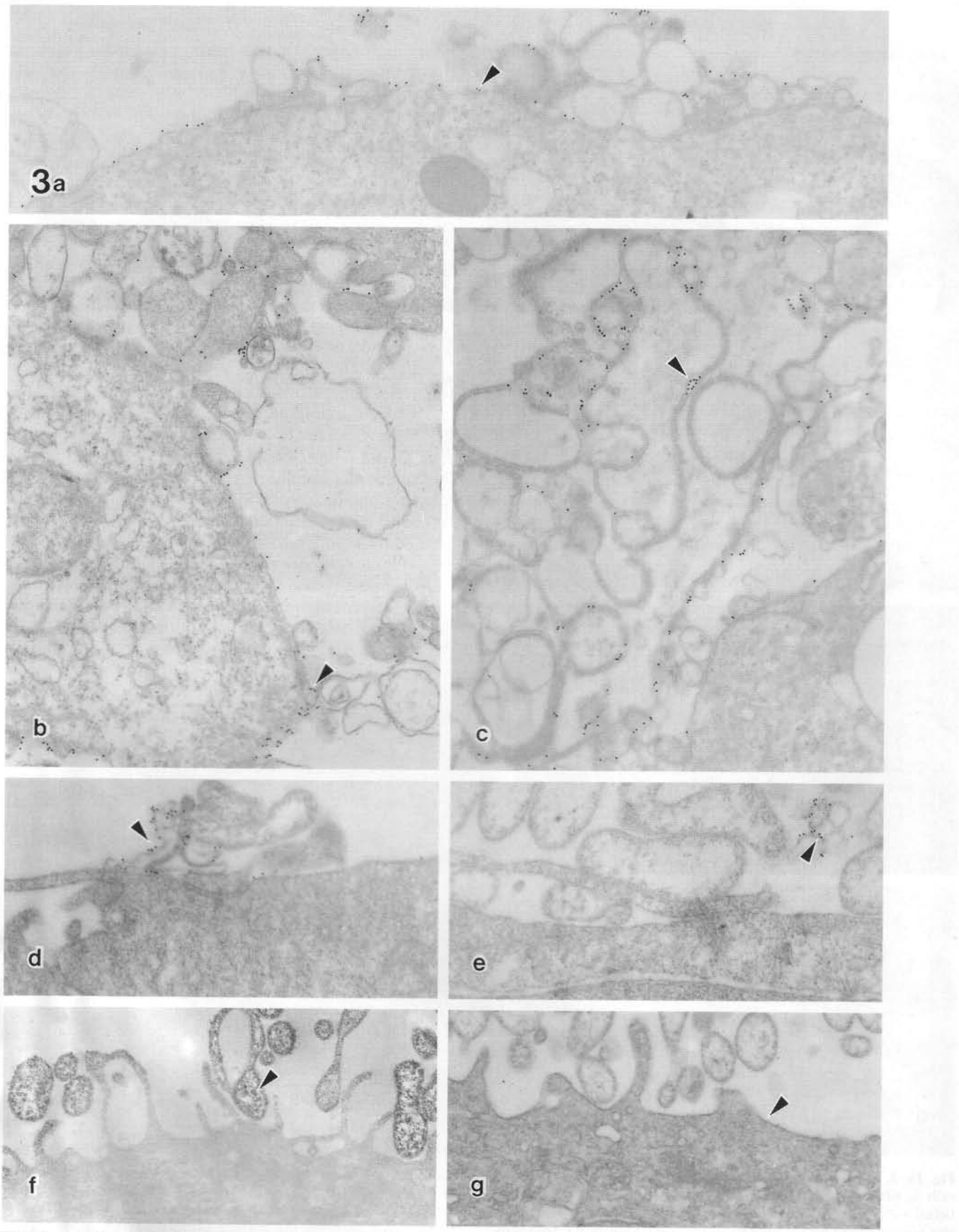

Fig. 3a–g. Effect of in situ enzyme digestion of CaSki cells on Mab 1H10 binding. Unfixed CaSki cells were digested with enzymes, as described in the Materials and methods, then immunolabelled with Mab 1H10 and Ab-gold (**a–e**) or Ab-PO (**f**, **g**), and examined by electron microscopy. Cells were digested with **a** lipase, **b** protease, **c** papain, **d** neuraminidase, **e** hyaluronidase, **f** chondroitinase ABC (25 min), or **g** chonroitinase (60 min). *Arrowheads* indicate the locations of immunoreactions. Note the absence of peroxidase activity after 60 min of chondroitinase digestion in **g**. Magnification: **a** ×33500; **b** ×26700; **c** ×33300; **d** ×26000; **e** 27800; **f, g** ×16700

Mab 1H10 binding to tissue sections

Mab binding to human tissue was tested on thin sections of normal and tumor samples, which were stored at −70° C until use. Cryostat sections (5 µm) of tissue were pretreated with acetone, incubated in 3% H_2O_2 in methanol to deactivate endogenous peroxidase activity and blocked with normal human serum [diluted 1:5 in phosphate-buffered saline (PBS)] to decrease nonspecific background activity. Antibody binding was assayed by the peroxidase anti-peroxidase technique (Johnstone and Thorpe 1987). Briefly, Mab 1H10 diluted to 50 µg/ml was incubated with tissues followed by reaction with rabbit anti-mouse IgG and mouse peroxidase anti-peroxidase conjugate; binding was visualized with 3,3′-diaminobenzidine. Any staining at intensities above the negative control background (control IgG_3 Mab ATCC CL189 reacting with influenza virus) was considered to be a positive result. The ability of Mab 1H10 to agglutinate blood-group antigens was assayed using normal red blood cells of groups A, B and O.

Characterization of 1H10 antigen by enzyme digestions

Membrane fractions were obtained from 10^8 viable CaSki cells by douncing cells in 5 ml of 1 m*M* $NaHCO_3$ containing 1 m*M* phenylmethylsulphonyl fluoride and 2 m*M* aprotinin and then centrifuging at $3000 \times g$ for 5 min to remove nuclei. Cells were centrifuged at $100000 \times g$ for 1 h and the pellet was sonicated on ice for 1 min. Enzyme digestion experiments were performed at 37° C for 1 h. Aliquots (100 µl) of membrane homogenates were incubated with an equal volume of each of the following: 0.01 units neuraminidase (type V, Sigma, St. Louis, Mo., USA) in 50 m*M* sodium acetate, pH 5.1; 0.05% trypsin (Sigma) in 40 m*M* *Tris*-HCl buffer, pH 8.1 with 50 m*M* $CaCl_2$; 0.05% chymotrypsin (Sigma) in 40 m*M* *Tris*-HCl, pH 8.1 with 50 mM $CaCl_2$; 5000 units of lipase (type VII-S, Sigma) in 50 m*M* *Tris*-HCl buffer, pH 7.6; 100 m*M* sodium periodate in 50 m*M* sodium acetate, pH 5.1; 0.05% chondroitinase ABC (Sigma) in 250 m*M* *Tris*-HCl, 176 m*M* $NaHCO_3$ and 250 m*M* NaCl at pH 8.0; 0.05% papain (Sigma) in 2 m*M* EDTA, 5 m*M* cysteine-HCl, pH 6.2; 0.1% hyaluronidase (Sigma) in 50 m*M* sodium acetate, pH 5.1; 0.05% protease (type XIV, Sigma) in 40 m*M* *Tris*-HCl, pH 7.8.

After enzymatic digestion, samples were mixed with an equal volume of Laemmli buffer (Laemmli 1970), separated by electrophoresis on a 3–12.5% gradient acrylamide gel and transferred onto a nitrocellulose membrane. Nitrocellulose membranes were blocked with 3% skim milk (Difco, Detroit, Mich., USA) in PBS. 1H10 antigen was identified by immunostaining with Mab 1H10 and goat anti-mouse IgG-peroxidase (PO) conjugate (Lee et al. 1988). Visualization of the binding of Mab 1H10 to antigen was then achieved by reaction with 4-chloro-1-naphthol. Sensitivity of the 1H10 antigen to diverse enzyme treatments has also been evaluated by immunofluorescence as previously described (Roffler et al. 1991 a).

Lectin binding and immuno-electron microscopy

CaSki cell monolayers were grown on Thermanox round plastic coverslips (Nunc, Kamstrup, Denmark). CaSki cells were firmly attached on the coverslips, therefore lectin binding, enzyme digestions and immunostaining were performed without loss of cells from the coverslip. Fixed and unfixed cell samples were incubated with Mab 1H10 [1/25–1/50 dilution of 1.37 mg/ml stock in Dulbecco's phosphate-buffered saline (D-PBS)] for 90 min with shaking at room temperature. This was followed by incubation with anti-mouse IgG-peroxidase (Ab-PO) or anti-mouse biotin conjugate and streptavidin-colloidal gold (Ab-gold) for the positive controls. Samples omitting Mab 1H10 before incubation with Ab-PO were used as negative controls.

For experiments examining the effects of in situ enzyme treatment on Mab 1H10 binding, unfixed CaSki cells were digested with enzymes in the buffers described above before incubation with Mab 1H10 and Ab-PO or Ab-gold. The ability of lectins to block Mab 1H10 binding to CaSki cells was also examined after first fixing cells in 2% glutaraldehyde in 0.1 *M* D-PBS, pH 7.2, for 30 min. Three kinds of lectin-PO conjugates were used at 1 mg/ml: concanavalin A (Con A-PO), wheat germ agglutinin (WGA-PO) and *Ricinus communis* agglutinin (RCA-PO; Sigma, St Louis). The lectin-PO conjugate (Wang et al. 1990) was incubated with fixed CaSki cells for 1 h at 20° C. After washing several times with D-PBS, cells were incubated with Mab 1H10 and Ab-gold. Peroxidase activity was shown by reaction with diaminobenzidine (DAB, 0.5 mg/ml) and hydrogen peroxide (0.04%) in 0.05 *M* *Tris*-HCl buffer, pH 7.6, for 15 min. The electron-dense deposits were formed by staining with 1% osmium tetroxide and the samples were then routinely processed for electron microscopy.

Quantitation of antibody binding to CaSki cell membrane

The intensity of peroxidase staining of CaSki cell membranes was quantified using a Molecular Dynamics computing densitometer. Images of electron micrograph film negatives were analysed (Wang 1989) using ImageQuant (Molecular Dynamics, Sunnyvale, Calif., USA) software. Between 30 and 100 scans perpendicular to the cell membrane were made for each film. The areas under the curves of peroxidase intensity versus distance across the cell membrane were integrated and mean intensities and standard errors were calculated from 2 to 4 films for each experimental condition. The results are expressed relative to CaSki cells incubated with Mab 1H10 and Ab-PO.

Results

Mab 1H10, a murine IgG_3 antibody, has been shown to bind to human cervical carcinoma tumors but not to normal human cells and tissues (Roffler et al. 1991 a). In this study, the biochemical nature of the antigen recognized by Mab 1H10 was investigated. Membrane homogenates from CaSki cervical carcinoma cells, which express the Mab 1H10 antigen, were digested with differ-

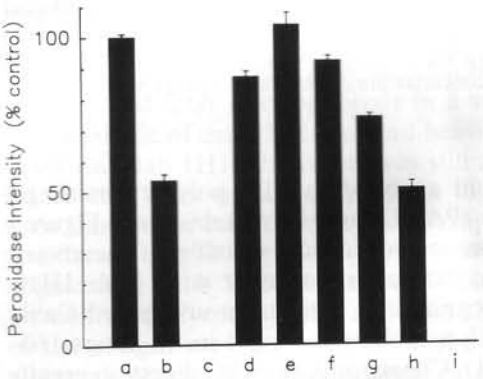

Fig. 4. Quantitation of intensity of peroxidase staining of CaSki cells. Electron micrographs of peroxidase-stained CaSki cells were imaged and scanned for intensity of membrane peroxidase staining as described in the Materials and methods. Mean intensities of membrane peroxidase are shown for Mab 1H10 and Ab-PO labelled CaSki cells that were previously **a** untreated (control), or pretreated with **b** chondroitinase for 25 min, **c** chondroitinase for 60 min, **d** neuraminidase, **e** hyaluronidase, **f** trypsin, **g** papain, **h** protease and **i** sodium periodate

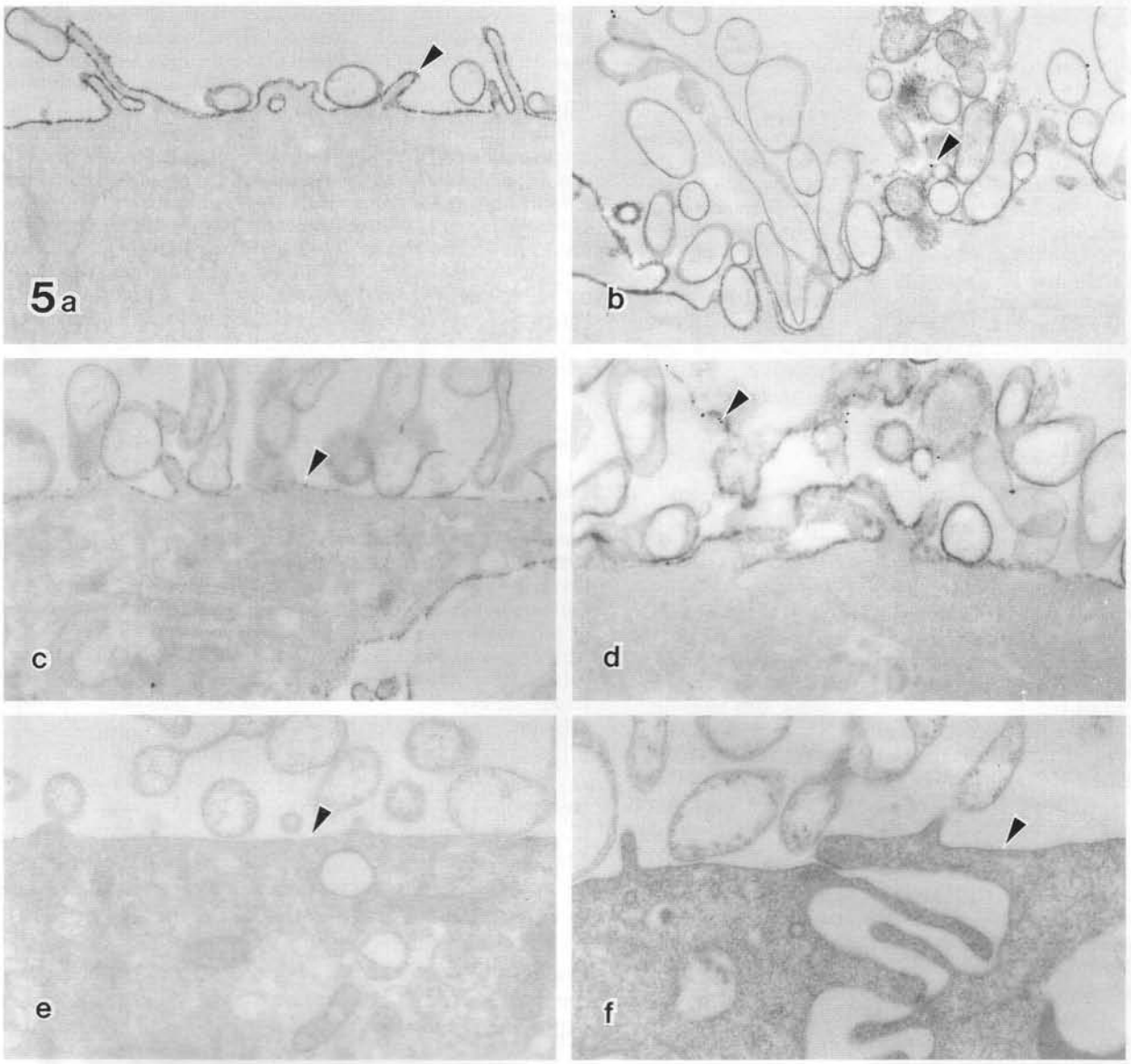

Fig. 5a–f. Effect of lectin blocking or sodium periodate treatment on Mab 1H10 binding to CaSki cells in situ. Cells were labelled with **a** wheat germ agglutinin-peroxidase complex (WGA-PO), **b** *Ricinus communis* agglutinin-peroxidase complex (RCA-PO) and 1H10-Ab-gold, **c** concanavalin A-peroxidase complex (Con A-PO), or **d** Con A-PO and 1H10-Ab-gold. Note the reduced binding of immuno-gold particles after RCA-PO binding (*arrowhead*). Sodium periodate treatment of CaSki cells blocked **e** WGA-PO and **f** Con A-PO binding (*arrowheads*). Magnification: **a** ×13800; **b** ×16600; **c** ×16700; **d** ×27600; **e** ×20700; **f** ×27600

ent enzymes and analysed by SDS-polyacrylamide gel electrophoresis (PAGE) and immunoblotting. Figure 1 shows the immunocytochemical staining of membrane components on nitrocellulose paper with Mab 1H10. Mab 1H10 recognized an antigen on undigested CaSki membranes with a molecular weight in the range 160–190 kDa (lane 1). Chondroitinase ABC digestion greatly reduced but did not eliminate Mab 1H10 binding to the major band at 175 kDa (lane 2). Neuraminidase, on the other hand, did not affect Mab 1H10 antigen binding (lane 3). Pretreatment of CaSki membrane homogenates with trypsin or chymotrypsin reduced the molecular weight of the antigen to 110 kDa (lanes 4 and 5). Digestion with papain or protease resulted in a broad smear from 180 to 75 kDa, which is characteristic of highly glycosylated proteins (lanes 6 and 7). Lipase digestion slightly decreased antibody binding (lane 8). Treatment of membranes with sodium periodate, in contrast, totally destroyed the epitope recognized by Mab 1H10 (lane 9).

The antigenicity of the 1H10 antigen in situ was examined using fixed and unfixed monolayer cells grown on a plastic coverslip or in cell suspension. Cells were pretreated with various enzymes, lectins or sodium periodate. Treated cells stained with Mab 1H10 followed by Ab-PO or Ab-gold conjugates were examined by electron microscopy. Three control groups, including unfixed CaSki cells labelled with Mab 1H10 and Ab-gold (Fig. 2a) and fixed cells labelled with Mab 1H10 and Ab-PO (Fig. 2b) or Mab 1H10 and Ab-gold (Fig. 2c) showed positive reactions. A negative control was ob-

tained by omitting Mab 1H10 before incubation of CaSki cells with Ab-PO (Fig. 2d).

The effect of enzymatic digestion of CaSki cells on Mab 1H10 binding in situ was examined by immunoelectron microscopy. Treatment of CaSki cells with lipase did not affect Mab 1H10 binding to antigen as shown by subsequent reaction with Ab-gold (Fig. 3a). Digestion of CaSki cells with protease or papain disrupted the structure of the cell membrane but Mab 1H10 binding occurred to a similar extent to Mab 1H10 binding to untreated CaSki cells (Fig. 3b, c). Removal of terminal sialic acid or hyaluronic acid residues from carbohydrates present on fixed CaSki cells, by digestion with neuraminidase or hyaluronidase, decreased but did not eliminate Ab-gold binding to cells (Figs. 3d, e). Chondroitinase digestion of CaSki cells for 25 min at 37° C eliminated binding of Mab 1H10 to the cell membrane but only reduced the binding to extracellular vesicles (Fig. 3f). However, increasing the time of chondroitinase digestion to 1 h totally eliminated Mab 1H10 binding to both the cell membrane and vesicles in situ (Fig. 3g). Quantitative data of peroxidase staining of CaSki cell membranes by scanning the film negatives of electron micrographs with a computing densitometer indicated that Mab 1H10 binding was also reduced most by pretreating CaSki cells with chondroitinase (Fig. 4b, c) and sodium periodate (Fig. 4i) compared with untreated controls (Fig. 4a). Parallel quantitative results are shown for neuraminidase and hyaluronidase (Figs. 4d, e) and trypsin, papain and protease digestions (Figs. 4f–h).

The ability of lectins to compete with Mab 1H10 for antigen expressed on CaSki cells was also examined (Table 2; Fig. 5). WGA-PO and RCA-PO conjugates strongly bound to CaSki cells (Fig. 5a, b) while Con A-PO bound only weakly (Fig. 5c). Preincubation of fixed CaSki cells with WGA-PO or RCA-PO partially blocked Mab 1H10 binding to cells as shown by decreased immunogold binding (Table 2; Fig. 5b). Con A-PO was less effective at blocking Mab 1H10-immunogold binding to the cell membrane (Fig. 5d). Oxidation of carbohydrates on the surface of CaSki cells with sodium periodate eliminated lectin binding to cells (Fig. 5e, f).

Table 2. Effect of lectin competition and periodate oxidation on Mab binding to CaSki cells in situ

Primary treatment or lectin	Antibody or lectin	Peroxidase intensity	Gold binding
None	1H10/Ab-gold	/	++
WGA-PO	1H10/Ab-gold	+	+/−
RCA-PO	1H10/Ab-gold	+	+/−
Con A-PO	1H10/Ab-gold	+/−	+
Sodium periodate	1H10/Ab-PO	−	/
Sodium periodate	1H10/Ab-gold	/	−
Sodium periodate	WGA-PO	−	/
Sodium periodate	RCA-PO	−	/

WGA-PO, wheat germ agglutinin-peroxidase complex; RCA-PO, *Ricinus communis* agglutinin-peroxidase complex; Con A-PO, concanavalin A-peroxidase complex; Ab-PO, anti-mouse IgG-peroxidase conjugate; Ab-gold, anti-mouse IgG biotin followed by streptavidin-gold complex

++, Strongly positive; +, positive; +/−, weakly positive; −, negative; /, no data

Discussion

Several monoclonal antibodies have been developed against human cervical tumors including carcinoma (Koprowska et al. 1985), adenocarcinoma (Koizumi et al. 1988), cytoplasmic antigens of intraepithelial neoplasia (Koprowska and Zipfel 1988) and benign epithelial metaplasia (Fray et al. 1984). Mab 1H10 recognizes a cell surface antigen expressed on human cervical carcinoma cells with molecular weight of ca. 160–190 kDa. Mab 1H10 reacted with 40/97 human cervical carcinoma samples without cross-reaction to normal human tissues and cells. The epitope identified by Mab 1H10 is sensitive to sodium periodate in vitro suggesting that the major binding site for Mab 1H10 is a carbohydrate (Roffler et al. 1991a).

Lectin binding to the terminal sugars of carbohydrates on cell surfaces has been widely used to identify cell differentiation during fetal development (Taatjes et al. 1990; Wang et al. 1990) and carcinogenesis (Byrne et al. 1989a, b; Zhang et al. 1989; Pancino et al. 1991). Enzymes specific for individual terminal monosaccharides have also been applied to help identify antibody recognition epitopes (Neri et al. 1976; Johnson et al. 1986; Sato et al. 1987; Plendl and Schumacher 1989; Wang et al. 1990). In this study, these techniques were combined to characterize the 1H10 antigen on CaSki human cervical carcinoma cells.

Enzymatic digestion experiments showed that digestion of CaSki membrane homogenates with trypsin or chymotrypsin reduced the molecular weight of the Mab 1H10 antigen from 180 to 110 kDa. Trypsin and chymotrypsin are relatively specific proteases, preferentially cleaving polypeptides at arginine and lysine or tyrosine, phenylalanine, tryptophan and methionine residues, respectively (Neurath and Schwert 1950). Digestion with papain or protease, which have broad substrate specificities, resulted after electrophoresis in a smear, which is characteristic of carbohydrates and heavily glycosylated proteins. Mab 1H10, however, was still able to bind to CaSki cells after papain or protease digestion in situ, even though the cell structure was disrupted by proteolytic treatment. This result indicates that the epitope recognized by Mab 1H10 is present on the carbohydrate portion of a glycoconjugate and that the protein core of the antigen may be anchored in the cell membrane where it is protected from proteolytic cleavage. Increased Mab 1H10 binding to CaSki cells after proteolytic digestion is suggested to result from removal of protein that can block antibody access to the carbohydrate epitope on the native antigen. The sensitivity of the 1H10 epitope to periodate oxidation further supports the hypothesis that the epitope is a carbohydrate. Lipase digestion of cell membrane homogenates did not affect the molecular weight of the 1H10 antigen although antibody binding

was slightly decreased. Mab 1H10 binding in vitro or in situ appeared to be resistant to lipase digestion of CaSki cells, leading to the conclusion that the 1H10 antigen is neither a lipoprotein nor a glycolipid. Mab 1H10 binding to CaSki cells was not greatly affected by neuraminidase or hyaluronidase treatment. It is thus suggested that sialic and hyaluronic acids are of minor importance in the recognition site or conformational structure of the Mab 1H10 epitope.

Chondroitinase ABC can remove components of chondroitin sulphate proteoglycans from cell surfaces (Johnson et al. 1986). The results of enzyme digestions in vitro and in situ indicate that the epitope recognized by Mab 1H10 is a chondroitin sulphate-containing molecule rather than a mucin.

The lectins and enzymes evaluated in this study were selected for their specificities to common sugar residues present on cell surfaces. WGA and RCA were found to bind strongly to CaSki cells while Con A bound more weakly. Preincubation of CaSki cells with these lectins showed that WGA-peroxidase and RCA-peroxidase conjugates were able partially to compete with Mab 1H10 for the surface antigen, indicating that the Mab 1H10 antigen may contain *N*-acetyl-galactosamine or *N*-acetyl-glucosamine residues at or near its epitope. Con A-peroxidase conjugate was less effective at preventing Mab 1H10-immunogold binding to CaSki cells, suggesting that mannose residues are not critical for Mab 1H10 binding to the epitope.

In conclusion, the tumor-associated antigen identified by Mab 1H10 appears to be a chondroitin sulphate proteoglycan containing a polypeptide of at least 70 kDa that can be removed by trypsin or papain digestion. Alterations in the glycoconjugate profile of neoplastic cells compared to normal cells have been well documented (Nicolson 1976; Zhang et al. 1989). Some human cervical tumors are suggested to express an altered glycoconjugate profile which can be detected by Mab 1H10; the altered profile may prove useful for detection or therapy of cervical carcinoma.

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References

Bischoff J, Lodish HF (1987) Two asialoglycoprotein receptor polypeptides in human hepatoma cells. J Biol Chem 262:11825–11832

Byrne P, Williams A, Rollason T (1989a) Studies of lectin binding to the human cervix uteri: I. Normal cervix. Histochem J 21:311–322

Byrne P, Williams A, Rollason T (1989b) Studies of lectin binding to the human cervix uteri: II. Cervical intraepithelial neoplasia and invasive squamous carcinoma. Histochem J 21:323–336

Dodgson J, Walker EM, Hussein KA, Robertson AJ, Duncan ID (1989) The increasing problem in Tayside of cervical cancer in younger women. Scot Med J 34:403–405

Fray RE, Husain OAN, To ACW, Watts KC, Lader S, Rogers GT, Taylor-Papadimitriou J, Morris NF (1984) The value of immunohistochemical markers in the diagnosis of cervical neoplasia. Br J Obstet Gynecol 91:1037–1041

Goldstein IJ, Hughes RC, Monsigny M, Osawa T, Sharon N (1980) What should be called a lectin. Nature 285:66

Johnson VG, Schlom J, Paterson AJ, Bennett J, Magnani JL, Colcher D (1986) Analysis of a human tumor-associated glycoprotein (TAG-72) identified by monoclonal antibody B72.3. Cancer Res 46:850–857

Johnstone A, Thorpe R (1987) Immunochemistry in practice, 2nd edn. Blackwell Scientific Publications, Oxford

Köhler G, Milstein C (1975) Continuous cultures of fused cells secreting antibody of predefined specificity. Nature 256:495–497

Koizumi M, Uede T, Shijubo N, Kudo R, Hashimoto M, Kikuchi K (1988) New monoclonal antibody, 1C5, reactive with human cervical adenocarcinoma of the uterus, with immunodiagnostic potential. Cancer Res 48:6565–6572

Koprowska I, Zipfel SA (1988) A monoclonal antibody that binds to tumor associated antigens of exfoliated cells in the smears of patients with cervical intraepithelial neoplasia. Acta Cytol 32:293–297

Koprowska I, Zipfel SA, Himes TR, Herlyn M (1985) Common antigenic sites on exfoliated cells derived from cervical carcinoma and in tumor cells of nonuterine origin as determined by monoclonal antibodies in immunoperoxidase assay. Cancer Res 45:5964–5968

Laemmli UK (1970) Cleavage of structural proteins during the assembly of bacteriophage T4. Nature 227:680–685

Lee N, Zhang SQ, Testa D (1988) A rapid multicolor Western blot. J Immunol Methods 106:27–30

Murphy MF, Campbell MJ, Goldblatt PO (1988) Twenty year's screening for cancer of the uterine cervix in Great Britian, 1964–84: further evidence for its ineffectiveness. J Epidemiol Community Health 42:49–53

Neri G, Giuliano MC, Capetillo S, Gilliam EB, Hixson DC, Walborg EF Jr (1976) Effect of neuraminidase and papain treatment on lectin-induced agglutination of Novikoff tumor cells and assay of lectin receptor activity of the glycopeptides released from the cell surface by papain. Cancer Res 36:263–268

Neurath H, Schwert GW (1950) The mode of action of the crystalline pancreatic proleolytic enzymes. Chem Rev 46:69–153

Nicolson GL (1976) Trans-membrane control of the receptors on normal and tumor cells. II. Surface changes associated with transformation and malignancy. Biochim Biophys Acta 458:1–72

Pancino G, Osinaga E, Charpin C, Mistro D, Barque JP, Roseto A (1991) Purification and characterization of a breast-cancer-associated glycoprotein not expressed in normal breast and identified by monoclonal antibody 83D4. Br J Cancer 63:390–398

Plendl J, Schönleber B, Schmahl W, Schumacher U (1989) Comparison of the unmasking of lectin receptors by neuraminidase and by enzyme-free buffer alone. J Histochem Cytochem 37:1743–1744

Roffler SR, Yu MH, Chen MR, Chen SC, Hsu CT, Yeh MY (1991a) New monoclonal antibody against human cervical carcinoma with diagnostic and therapeutic potential. Chinese J Microbiol Immunol 24:19–29

Roffler SR, Yu MH, Chen BM, Tung E, Yeh MY (1991b) Therapy of human cervical carcinoma with monoclonal antibody-*Pseudomonas* exotoxin conjugates. Cancer Res 51:4001–4007

Russell WC, Newman C, Williamson DH (1975) A simple cytochemical technique for demonstration of DNA in cells infected with mycoplasmas and viruses. Nature 253:461–462

Sato N, Yagihashi A, Okubo M, Torigoe T, Takahashi S, Sato T, Kikuchi K (1987) Characterization of tumor rejection antigen molecules of chemically induced murine colon tumor C-C26. Cancer Res 47:3147–3151

Sheer DG, Schlom J, Cooper HL (1988) Purification and composition of the human tumor-associated glycoprotein (TAG-72) de-

fined by monoclonal antibodies CC49 and B72.3. Cancer Res 48:6811–6818

Swanson GM, Belle SH, Young JL (1983) U.S. trends in carcinoma of the cervix: incidence, mortality and survival. In: Hafez ESE, Smith JP (eds) Carcinoma of the cervix: biology and diagnosis. Martinus Nijhoff, Netherlands, pp 1–9

Taatjes DJ, Barcomb LA, Leslie KO, Low RB (1990) Lectin binding patterns to terminal sugars of rat lung alveolar epithelial cells. J Histochem Cytochem 38:233–244

Wang JJ (1989) Immunocytochemical demonstration of the binding of growth-related polypeptide hormones on chick embryonic tissues. Histochemistry 93:133–141

Wang JJ, Yin CS, Teng CS (1990) Lectin bindings and diethylstilbestrol effects on the recognition of Müllerian inhibiting substance (MIS) on chick Müllerian ducts by MIS-antiserum. Histochemistry 95:55–62

Yeh MY, Roffler SR, Yu MH (1992) Doxorubicin-monoclonal antibody conjugate for therapy of human cervical carcinoma. Int J Cancer 51:274–282

Yeh S (1985) Survey of cancer in Taiwan. In: Chang EH, Lin JK, Huang PC (eds) Molecular biology of neoplasia. Academia Sinica, Taipei, pp 23–31

Zhang SM, Wu M, Chen H, Zhang X (1989) Changes of glycoconjugates in human hepatocellular carcinoma. Histochemistry 92:171–175