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New Monoclonal Antibody Against Human Cervical Carcinoma with Diagnostic and Therapeutic Potential

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A new murine monoclonal IgG_3 antibody (Mab 1H10) was developed with specificity for human cervical carcinoma and several other tumor types. Antibody reactivity against a panel of tumor cell lines was examined by indirect immunofluorescence and quantified by flow cytometry. Mab 1H10 reacted with cervical, colorectal and bladder carcinoma cells and to a lesser extent melanoma and hepatocellular carcinoma cells but did not react with human fibroblasts, lymphocytes or RBCs. Mab 1H10, as assessed by immunohistochemical staining, bound 40/97 cervical carcinoma tissue samples, 8/16 colorectal carcinoma samples as well as a population of osteogenic sarcoma and lung, ovarian and bladder carcinoma tissues. Mab 1H10 did not react with any normal tissue or cell samples tested including cervix, ovary, breast, liver, colon, bladder, lung, spleen, cerebrum, lymphocytes or RBCs. Mab 1H10 may be useful for the targeting of drugs, toxins or radioisotopes to cervical carcinoma in humans.

Key words: ***Monoclonal antibody, Cervical carcinoma.***

Cervical carcinoma is one of the common lethal malignancies affecting women around the world. Despite the widespread use of cervical cytologic screening programs, it is still a serious problem[1,2,3]. In Taiwan, 49.9% of 23620 primary malignant tumors from females diagnosed by surgical examinations from 1964 to 1983 were found to be carcinoma of the uterine cervix[4]. In another study, the age-adjusted cancer mortality rate of cervical carcinoma in China between 1973 and 1975 was 10.0/100,000 females, ranking second behind stomach cancer at 10.2 deaths per 100,000[5]. These high incidence and mortality rates indicate that cervical carcinoma is a serious problem in the Republic of China and that improved methods of detection

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Mab 1H10 antigen characterization. Membranes from 10^8 viable CaSki cells were obtained by first douncing cells in 5 mls of 1 mM $NaHCO_3$ containing 1 mM PMSF and 2 mM aprotinin followed by centrifugation at 3000 g for 5 minutes to remove nuclei. After pelleting at 100,000 g for 1 hour and sonicating on ice for 1 min, 100 μl aliquots of membranes were incubated at 37°C for 1 hour with an equal volume of each of the following mixtures: 0.01 units neuraminidase in 50 mM sodium acetate, pH 5.1; 0.05% trypsin in 40 mM Tris-HCl, pH 8.1 with 10 mM $CaCl_2$; 5000 units lipase in 50 mM Tris-HCl, pH 7.6 or 100 mM sodium periodate in 50 mM sodium acetate, pH 5.1. Samples were then mixed with an equal volume of Laemmli buffer[23], electrophoresed on a 3-12.5% gradient acrylamide gel and transferred to a nitrocellulose membrane. After blocking with 3% skim milk (Difco) in PBS, antigen was identified by immunoblotting with Mab 1H10 and a goat anti-mouse IgG—HRP conjugate[24].

Sensitivity of the antigen to treatments was also evaluated by immunofluorescence. 10^6 CaSki cells were incubated with the indicated concentrations of trypsin or lipase in PBS or neuraminidase or sodium periodate in 50 mM acetate, 100 mM NaCl, pH 5.0 at 37°C for 1 hour. After treatment, cells were washed three times with PBS and Mab 1H10 binding was measured by indirect immunofluorescence. Results are reported as mean fluorescent intensities, calculated as described above.

RESULTS

Mab 1H10 was generated by fusing NS-1 mouse myeloma cells with splenocytes of mice that had been immunized with CaSki human cervical carcinoma cells. Ascites-produced Mab had a purity of 85-90% while culture-produced Mab had a purity greater than 95% as determined by SDS PAGE under reducing and non-reducing conditions. Mab 1H10 is an IgG_3 antibody with an isoelectric point of 8.5 for the whole antibody and 9.1 for the $F(ab')_2$ fragment.

Mab reactivity with tumor cell lines. The reactivity of Mab 1H10 with a panel of human tumor cell lines was investigated by indirect immunofluorescence and quantified by flow cytometry (Table 1). Mab 1H10 strongly reacted with human cervical carcinoma cell lines CaSki and ME180 and to a lesser degree with RT4 bladder carcinoma cells and HT29 and SW1116 colon carcinoma cells. Mab 1H10 reacted moderately with several cell lines including SiHa, CC7T, LS 174T, TSGH 8301, PC-3, H 2823 and H 8447 cells. Antibody binding appeared limited to the cell membrane as determined by microscopic observation of immunofluorescent staining and electron microscopic examination of fixed cells reacted with Mab

Table 1. Reactivity of Mab 1H10 with human cell lines by immunofluorescene[a]

Cell line	Mean fluorescent intensity
Cevical carcinoma	
CaSki	142±3.4
ME-180	125±8.0
SiHa	53±3.8
CC7T	53±6.3
HS 1025	29±9.0
HS 1023	16±5.6
TSGH 8302	11±8.1
HeLa-M	10±1.1
Colon carcinoma	
HT-29	93±4.6
SW 1116	88±6.8
LS 174T	51±12
SW 480	30±2.7
COLO 205	14±4.8
HCT 116	1±0.1
H 3347	1±0.1
Bladder carcinoma	
RT 4	100±9.3
TSGH 8301	42±14
Prostate carcinoma	
PC-3	39±4.8
Laryngeal carcinoma	
HEp-2	2±0.8
Lung carcinoma	
H 928	5±0.8
H 2981-B	1±0.2
Liposarcoma	
HS 1079	12±5.4
Melanoma	
H 2823	69±11
H 1447	52±4.9
H 2682	40±1.0
H 2484-B	21±17
H 2669	19±4.9
H 2995	12±8.0
Hepatoma	
HA 22T	32±18
PLC	28±21
Hep 3B	14±12
Hep G2	5±3.2
Amniotic fibroblast	
WISH	1±0.5
Fibroblast	
HEL	1±0.5
FS	1±0.6

[a] 10,000 viable cells were reacted with 50 μg/ml Mab 1H10 or control IgG_3 and anti-mouse IgG FITC and measured by flow cytometry. Background immunofluorescence was subtracted from test antibody fluorescece before mean intensities were calculated as described in "Materials and Methods". Results represent the average of at least two independent experiments. Standard errors of the mean are also listed.

Table 2. Immunohistochemical reaction of Mab 1H10 with human cells and tissues[a]

Carcinoma	Positive/tested
Cervical carcinoma	40/97
Ovarian carcinoma	1/3
Endometrial carcinoma	0/2
Breast carcinoma	0/7
Hepatoma	0/6
Colorectal carcinoma	8/22
Bladder calcinoma	2/12
Renal cell calcinoma	0/1
Lung calcinoma	1/5
Prostate calcinoma	0/4
Testicular calcinoma	0/5
Stomach calcinoma	1/1[b]
Osteogenic sarcoma	1/1
Glioma	0/3
Meningioma	0/3
Astrocytoma	0/2

Normal	Positive/tested
Cervix	0/24
Ovary	0/1
Endometrium	0/2
Breast	0/4
Liver	0/14
Colon	0/4
Bladder	0/2
Kidney	0/2
Lung	0/3
Urethra	0/1
Thyroid	0/2
Esophagus	0/1
Cerebrum	0/3
Spleen	0/8
Lymphocytes	0/5
RBC (A)	0/5
RBC (B)	0/4
RBC (O)	0/4

[a] Reaction of Mab 1H10 with thin frozen tissue sections was determined by the peroxidase anti-peroxidase technique (22). Positive reaction was determined by comparison with control IgG_3 background staining. Antibody binding to RBCs and lymphocytes was examined by hemagglutination and immunofluorescence assays respectively.

[b] Weak reaction.

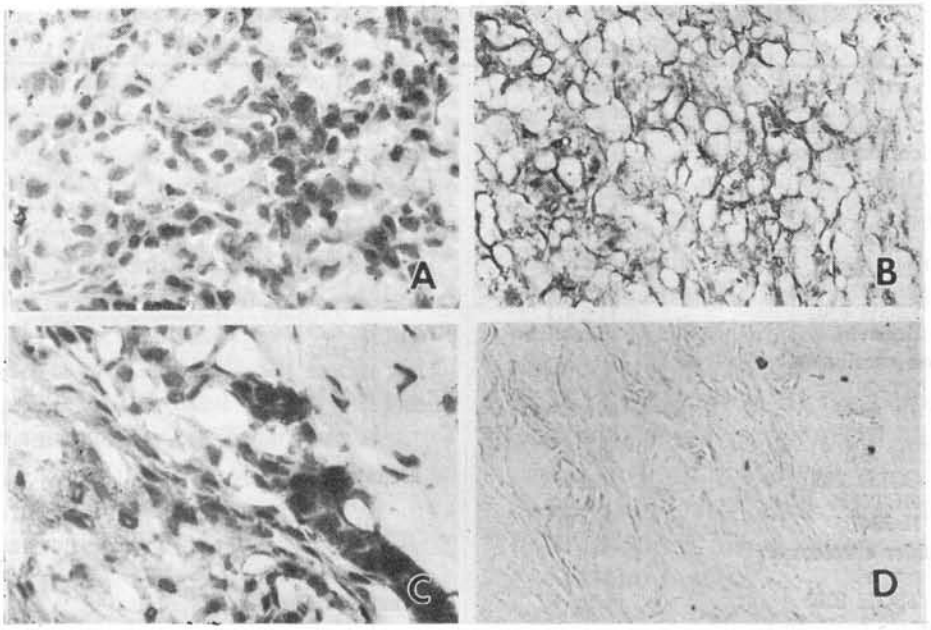

Fig. 1. Reactivity of Mab 1H10 with human cervical tissue samples. Thin sections of human cervical carcinoma (A, B) and normal cervix (C, D) were stained with hematoxylin (A, C) or reacted with Mab 1H10 and visualized by immunoperoxidase staining (B, D).

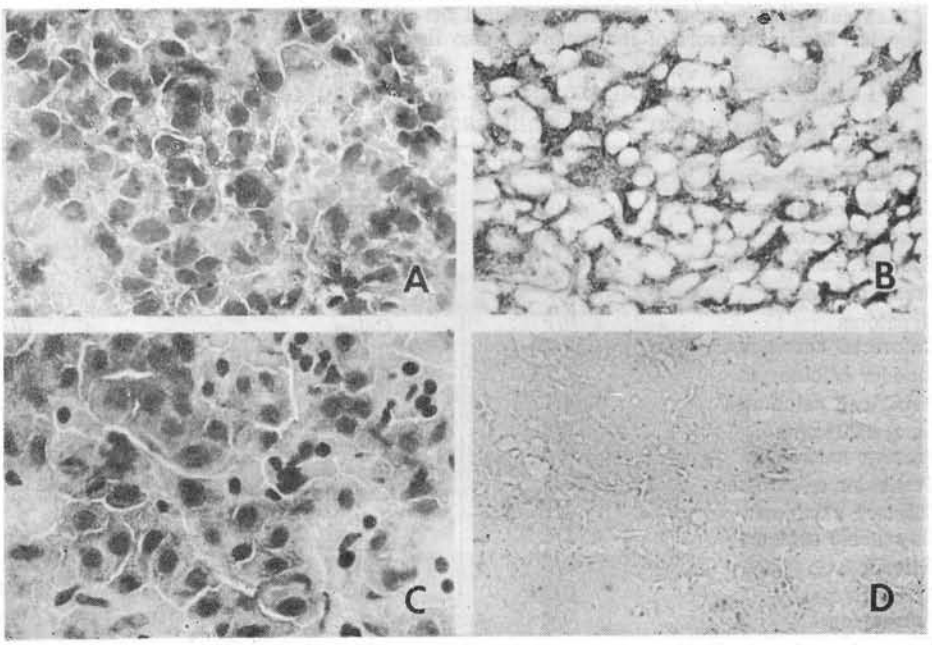

Fig. 2. Reactivity of Mab 1H10 with human tissue samples. Thin sections of human colon carcinoma (A, B) and normal liver (C, D) were stained with hematoxylin (A, C) or reacted with Mab 1H10 and visualized with immunoperoxidase staining (B, D).

1H10 and immunoperoxidase (data not shown). Reaction with other cell lines was weak or negative. Mab 1H10 did not bind to amniotic or fibroblast cell lines tested.

Mab binding to human tissues and cells. Table 2 summarizes results of Mab 1H10 reactivity with human tissues and cells as measured by PAP immunostaining or immunofluorescence. Mab 1H10 reacted with 40 out of 97 (40%) cervical carcinoma tumor samples but did not react with normal cervical tissue (Fig. 1). Mab 1H10 also reacted with some osteogenic sarcoma, colorectal, bladder, ovarian and lung carcinoma tissues but did not react with normal human cells or tissues tested (Table 2 and Fig. 2).

Characterization of Mab 1H10 antigen. Mab 1H10 antigen was characterized by treating CaSki cell membranes with sodium periodate or enzymes and

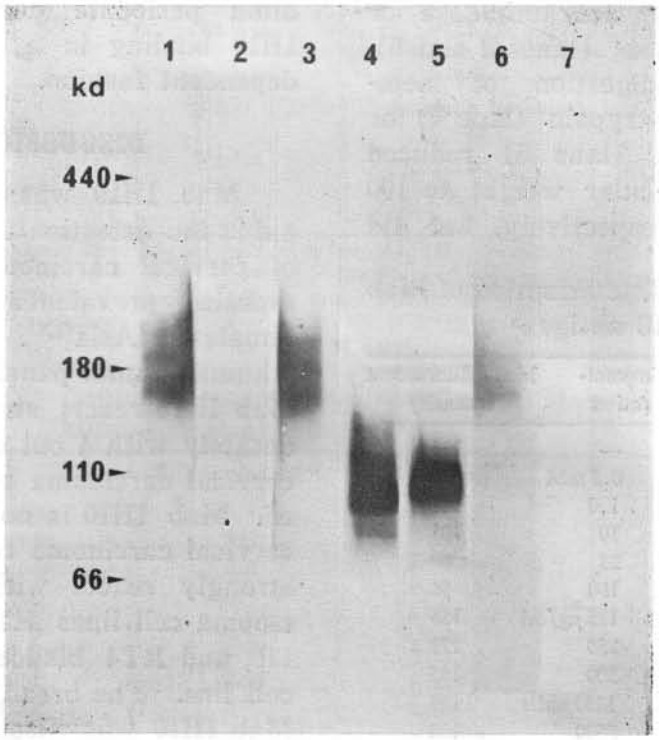

Fig. 3. SD PAGE and Western blot analysis of the Mab 1H10 antigen. Membranes from CaSki cervical carcinoma cells (lanes 1-6) or WISH fibroblasts (lane 7) were pretreated, electrophoresed in a gradient (3-12.5%) SDS gel, transferred to nitrocellulose paper and probed with Mab 1H10. Mab 1H10 binding was visualized with anti-mouse IgG peroxidase conjugate and reaction with 4-chloro-1-naphthol. CaSki membranes were: lane 1, untreated; lane 2, pretreated with sodium periodate; lane 3, pretreated with neuraminidase; lane 4, pretreated with trypsin; lane 5, pretreated with chymotrypsin; lane 6, pretreated with lipase. Lane 7 shows untreated WISH fibroblast membranes. Standard protein molecular weights (kd) are indicated on the left.

observing the effect on antigen migration in SDS gradient gels after transfer to nitrocellulose paper and reaction with Mab 1H10. Figure 3 shows that untreated CaSki cells primarily express 1H10 antigen as two bands with molecular weights of 160 and 190 kd (lane 1). Minor bands at 115 and 215 kd are also visible. Mab 1H10 binding was totally eliminated by treating antigen with sodium periodate (lane 2). In contrast, antigen was unaffected by neuraminidase or lipase treatment (lanes 3 and 6). Proteolytic digestion of membranes with trypsin (lane 4) or chymotrypsin (lane 5) reduced antigen molecular weight to 100 and 105 kd respectively, but did not decrease Mab 1H10 binding to antigen. Mab 1H10 did not bind to electrophoresed membranes of antigen-negative WISH fibroblasts (lane 7).

Table 3. Characterization of Mab 1H10 antigen[a]

Reagent	Concentration	Mean fluorescent intensity
Untreated	—	153
$NaIO_4$	0.5 mM	165
	1.0	147
	10	101
	25	69
	100	52
Trypsin	125 μg/ml	156
	250	171
	500	155
Lipase	1250 units	129
	2500	133
	5000	147
Neuraminidase	0.25 units/ml	159
	0.50	171
	1.00	161

[a] 10^6 viable CaSki cells were incubated with the indicated reagents for 1 hour at 37°C and Mab 1H10 binding to cells estimated by indirect immunofluorescence and quantified by flow cytometric analysis of 10^4 cells. Mean fluorescent intensities were calculated as described in "Materials and Methods".

These results were verified by measuring the ability of Mab 1H10 to bind viable CaSki cells after treatment with sodium periodate, trypsin, lipase or neuraminidase. Table 3 shows that Mab 1H10 binding to CaSki cells was unaffected by trypsin, lipase and neuraminidase but that sodium periodate decreased Mab 1H10 binding in a concentration dependent fashion.

DISCUSSION

Mab 1H10 was developed to aid in the detection and treatment of cervical carcinoma, a cancer especially prevalent among Chinese females in Asia[4,5]. Screening of a human tumor panel showed that Mab 1H10 reacts strongly to moderately with 4 out of 8 cervical cervical carcinoma cell lines tested. Mab 1H10 is not specific for cervical carcinoma cells, but also strongly reacts with colon carcinoma cell lines HT-29 and SW 116, and RT4 bladder carcinoma cell line. The broad reactivity of Mab 1H10 was also seen among human tumor tissue samples. Besides reacting with 40/97 human cervical carcinoma tissue samples, Mab 1H10 also reacted with some samples of osteogenic sarcoma and colon, bladder, ovarian and lung carcinoma tissues. Mab 1H10 may be useful for the detection and treatments of several kinds

of cancer in addition to cervical carcinoma. To be useful, antibody binding should be restricted to tumor tissues. Mab 1H10 did not bind to normal tissues tested. Mab 1H10 thus appears to recognize a cell surface antigen with expression limited to tumor cells.

The antigen recognized by Mab 1H10 is expressed on the membrane of CaSki cells and appears as two major bands in SDS acrylamide gels with molecular weights of 160 and 190 kd. The antibody recognition site appears to be a carbohydrate as suggested by the sensitivity of the antigen to sodium periodate treatment. Limited proteolytic digestion of antigen-containing membranes decreased the size of the antigen but did not affect antibody binding, suggesting that the antigen recognized by Mab 1H10 contains protein but that the epitope is not a protein. Mab 1H10 thus appears to recognize a carbohydrate moiety of a glycoconjugate present on the membrane of some tumor cells. The exact nature of the Mab 1H10 antigen is the subject of current investigation.

Other investigators have generated monoclonal antibodies reactive with cervical carcinoma. Mab 1H10, however, appears to differ from these antibodies. For example, Mab 1C5[25] reacts with human cervical adenocarcinoma rather than with squamous cervical carcinoma, Mab CE 407[16] bound a cytoplasmic antigen and Mabs HMFG-1 and HMFG-2[13] stain both cell membranes and cytoplasm and react with benign epithelium metaplasia. Koprowska and colleagues[15] examined several antibodies that react with cervical carcinoma. Mabs 99-57 and 31-74 react with cytoplasmic antigens and polymorphonuclear leukocytes, Mabs ME 491 and 73.3 recognize smaller antigens than does Mab 1H10 and Mab 57.1 reacted with only 1 out of 6 cervical carcinoma tissues. Many of the antibodies that react with cervical carcinoma also react with normal tissue, limiting their usefulness *in situ*[7-11].

Mab 1H10 may play a role in the detection and treatment of cervical carcinoma due to its specificity for tumor tissues. We have previously shown that Mab 1H10 labeled with ^{131}I can specifically localize in human cervical carcinoma tumors xenografted in nude mice (unpublished data), indicating the potential of using this antibody for the identification of cervical carcinoma tumors *in situ*. In addition, a conjugate formed by linking *Pseudomonas* exotoxin to Mab 1H10 was able to suppress the growth of solid human cervical carcinoma tumors in nude mice (unpublished results) suggesting that this antibody may be effective in targeting cytotoxic agents to tumors.

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對抗人類子宮頸癌細胞的新單源抗體

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本實驗利用融合瘤技術，針對國人婦女罹患率高的子宮頸癌，發展出單源抗體 1H10。該抗體與各種人類腫瘤及正常細胞株之反應性經間接免疫螢光染色及細胞計流儀之螢光定量，顯現 1H10 除了對子宮頸癌反應較强外，對部份大腸癌及膀胱癌亦具反應，對黑色素細胞瘤及肝癌則反應較弱。於正常纖維母細胞，淋巴球及紅血球則無反應。組織切片的免疫化學染色結果顯示，1H10 與 97 例中 40 例的子宮頸癌組織反應，16 例中 8 例的大腸癌組織反應，其他尚包括與部份的肺癌、卵巢癌及膀胱癌等組織反應。對所測試之各種不同正常組織，則無明顯的反應。藉由單源抗體 1H10 對子宮頸癌辨識的特異性，我們將進一步探究其與化學抗癌藥物、毒素或放射性同位素結合以用於人類子宮頸癌導向治療的可行性，從而將單源抗體推進臨床實用的領域。