HMMC-1: A Humanized Monoclonal Antibody With Therapeutic Potential Against Müllerian Duct-Related Carcinomas

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ABSTRACT

Purpose: The purpose of this research was to generate a human monoclonal antibody specific to gynecological cancers and to evaluate such an antibody as therapy for gynecological cancers.

Experimental Design: Transchromosomal KM mice were immunized with the human uterine endometrial cancer cell line SNG-S. Hybridomas were constructed between spleen cells from KM mice and mouse myeloma cells. Reactivity of the antibody was evaluated by immunohistochemistry of pathological specimens of gynecological cancers. Cytotoxicity of HMMC-1 against SNG-S cells was tested by in vitro cytotoxicity assays. The epitope of HMMC-1 was determined by transfection with a panel of glycosyltransferase cDNAs and by inhibition assays with chemically synthesized oligosaccharides.

Results: HMMC-1 is a human IgM monoclonal antibody that reacts positively with müllerian duct-related carcinomas with positive rates of 54.6% against uterine endometrial adenocarcinoma, 76.9% against uterine cervical adenocarcinoma, and 75.0% against epithelial ovarian cancer. HMMC-1 does not react with normal endometrium at proliferative or secretory phases, normal uterine cervix, or normal and malignant tissue from other organs, whereas it reacts weakly with the epithelium of the gall bladder and the collecting duct of the kidney. HMMC-1 exhibits antigen-dependent and complement-mediated cytotoxicity. Upon co-transfection with cDNAs encoding two glycosyltransferases required for fucosylated extended core O-glycan, mammalian cells express HMMC-1 antigen. Finally, binding of HMMC-1 to SNG-S cells is inhibited by synthetic Fucα1→2Galβ1→4GlcNAcβ1→3Galβ1→3GalNAcα1-octyl.

Conclusions: These results indicate that HMMC-1 specifically recognizes a novel O-glycan structure. The unique specificity and cytotoxicity of HMMC-1 strongly suggest a therapeutic potential of this antibody.

INTRODUCTION

Apical surfaces of epithelial cells exhibit a variety of carbohydrates attached to membrane proteins and lipids. When epithelial cells are transformed to carcinoma, cell surface glycoconjugates are significantly altered (1). In the last 2 decades, many monoclonal antibodies have been produced from spleen cells of mice immunized by carcinomas. Remarkably, many monoclonal antibodies produced by this procedure were directed to specific carbohydrate structures expressed on cancer cell surfaces (2). Such murine monoclonal antibodies with defined specificity have been used to determine the prognosis of cancer patients (3–5). However, murine antibodies cannot be used on cancer patients for therapeutic purposes because of their immunologic incompatibility. Thus, humanized monoclonal antibodies are required in the clinical arena.

Attempts to generate humanized monoclonal antibodies have been made by generating cross-species hybridomas, as exemplified by our antibody HMST-1 raised against endometrial adenocarcinoma (6). However, this method has a major obstacle: hybrid cells between human lymphocytes and mouse myelomas survive poorly in culture. Alternatively, recombinant mouse-human chimeric monoclonal antibodies have been produced, as exemplified by Trastuzumab (7) against breast cancer and Rituximab (8) against non-Hodgkin’s lymphoma. However, generation of chimeric monoclonal antibodies is still laborious, preventing rapid production of humanized monoclonal antibodies.

Recently, Tomizuka et al. (9, 10) succeeded in producing a transchromosomal KM mouse line in which human chromosomes 2 and 14 are stably introduced. Thus, these KM mice express the complete human immunoglobulin heavy chain and light chain (11). We immunized KM mice with SNG-S cells, a human uterine endometrial cancer cell line established in our laboratory (6, 12), and produced a human monoclonal antibody against SNG-S cells. This antibody, termed HMMC-1 (human...
monoclonal antibody against müllerian duct-related carcinoma),
reacts specifically with carcinomas of müllerian duct origin.
Furthermore, we found that HMMC-1 reacts specifically to a
novel mucin type O-glycan carbohydrate structure and exhibits
HMMC-1 antigen-dependent and complement-mediated cyto-
toxicity. These findings strongly indicate the potential utility of
HMMC-1 in clinical therapies of these gynecological mali-
gnancies.

MATERIALS AND METHODS

Cell Culture. SNG-S and SNG-II cells were cultured in
a 1:1 mixture of Dulbecco’s modified Eagle medium and Ham’s
F 12 medium (Life Technologies, Inc., Grand Island, NY)
supplemented with 10% fetal calf serum. SNG-S has been
established as a line that reacts strongly with the antiuterine
diagram and cervical tissue monoclonal anti-Lewis B antibody MSN-1
from the SNG-II line (12, 13).

Immunization of KM Mice and Screening of Hybrid-
mas. SNG cells were injected every 2 weeks (1 × 10⁷
cells/injection) into cross-bred KM mice. Spleen cells from
immunized mice were fused to Sp2/0-Ag14 mouse myeloma
cells (American Type Culture Collection No. CRL-1581) using
S-clone cloning medium (Sankoh Jyunyaku Co., Tokyo, Japan).
Hybridoma Cloning Factor (IGEN International, Inc., Gaithers-
burg, MD), HAT (ICN Biochemicals, Costa Mesa, CA), and HT
media (ICN Biochemicals). Clone positives for SNG-S cells and
negative for the control SKG-IIIA cells, derived from squamous
cervical cell carcinoma, were selected by screening culture
supernatants by ELISA (14). The positive hybridoma clones
were additionally verified by immunohistochemistry with hu-
manserine endometrial adenocarcinoma specimens. All of the
animals were maintained and handled in accordance to the rules
and regulations established by the Animal Care Committee of
School of Medicine, Keio University.

Tissue Preparation and Immunohistochemistry. For-
malin-fixed, paraffin-embedded specimens of normal and ma-
lignant human tissues were selected from the pathology files of
the Department of Obstetrics and Gynecology and the Division of
Diagnostic Pathology, School of Medicine, Keio University.
Three-micrometer sections were used for immunohisto-
chemistry. Specimens were blocked with goat F(ab’)2 fragment
to human κ chain (bound; Cappel, Aurora, OH) followed by incub-
ation with the hybridoma culture supernatant (5 µg HMMC-
1/mL). Biotin-conjugated goat antihuman κ chain and peroxi-
dase-conjugated avidin (ABC kit; Vector Laboratories,
Burlingame, CA) were used. Counterstaining was performed
using hematoxylin. Staining patterns in tissue specimens were
evaluated by staining intensity and frequency of positive cells.
Staining intensity was graded on an arbitrary scale of +, + + , or
+ + + + , whereas frequency was classified as <10%, 10% to
50%, and >50%, based on the percentage of positive glandular
or carcinoma cells in each section. Combining both intensity and
frequency, the reactivity of each specimen was categorized as
weak, moderate, or strong positive (15).

Complement-Mediated Cytotoxicity Assay. SNG-S
cells and SNG-II cells were grown in 96-well plates. Cells were
reacted with HMMC-1 hybridoma supernatant at 4°C for 1 hour.
Antibody sensitized cells were washed with cold gelatin veronal
buffer followed by incubation with diluted (1:5 to 1:40) active
human serum complement or C6-depleted inactive complement
(Sigma, St. Louis, MO) at 37°C for 30 minutes. A supernatant
of the cells was subjected to a lactate dehydrogenase assay using
Cytotox96 kit (Promega, Madison, WI).

Western Blot Analysis. SNG-S cells were cultured in
60-mm plates for 24-hours in medium containing 0, 5, and 10
mmol/L benzyl N-acetyl α-galactosaminide (Sigma), which inhib-
its glycosylation of O-linked glycoproteins (16). SNG-S cells
were lysed with 50 mmol/L Tris-HCl buffer (pH 8.0), contain-
ing 150 mmol/L NaCl, 0.1% SDS, 0.5% sodium deoxycholate,
1% Triton X-100, and 1 mmol/L phenylmethylsulfonylfluoride,
and the extract was applied to Western blot analysis with HMMC-1
antibody. For SDS-PAGE, proteins were resolved by electrophore-
sis on a Multi SDS-PAGE Gel 10/20 (Daiichi Pure Chemicals,
Tokyo, Japan) and were transferred to phenylmethylsulfonylflu-
ouride membrane (Millipore, Bedford, MA). The membrane was
soaked in PBS (pH 7.4) containing Block Ace Powder (Dainippon
Pharmaceutical, Osaka, Japan) and 0.05% Tween 20. The mem-
brane was reacted with diluted monoclonal antibody in blocking
solution (PBS containing 2% skim milk) for 2 hours at room
temperature. The membrane was then reacted with horseradish
peroxidase-conjugated goat antihuman IgM (Kierkegaard and
Perry Laboratories, Gaithersburg, MD) for 1 hour at room temper-
ature. Immunoreactive bands were detected using the enhanced
chemiluminescence reagent (American Biosciences, Bucking-
hamshire, United Kingdom).

Transfection and Immunocytochemistry. Mammalian
expression vectors for α1, 3/4 fucosyltransferase (17), core β
N-acetylgalactosaminyltransferase (18), core 2 β N-acetylgalac-
tosaminyltransferase-L (19), core 2 β N-acetylgalactosaminy-
transferase-M (20), and core 1 β1, 3-N-acetylgalactosaminyltrans-
ferase (21) were kindly provided by Drs. Junya Mitoma and Hiroto Kawashima at
the Burnham Institute. An expression vector for α1,2-fucosyltrans-
ferase-1 (22) was kindly provided by Dr. Assou El Battari, Glyco-
biologie et thérapies antitumorales, INSERM (Marseille, France).
The cDNA encoding galactosyltransferase-5 (23) for synthesis
of blood group type 1 structure was kindly provided by Dr. Hisashi
Narimatsu, Institute Molecular and Cellular Biology, Tsukuba
(Ibaraki, Japan).

SNG-II, COS, and CHO cells were grown on glass cover-
slips and transfected with cDNAs using LipofectAMINEPlus
(Invitrogen, Carlsbad, CA). Two days after transfection, expres-
sion of HMMC-1 antigen was examined by immunocyto-
chemistry using Histostain-SP kit (Zymed, South San Francisco, CA).

HMMC-1 Inhibition Assay with Synthetic Oligosac-
charides. Synthetic oligosaccharides Galβ1→4GlcNAcβ1→
3Galβ1→3GalNAcα1→octyl (extended core 1 O-glycan), and
Galβ1→4(Fucα1→3)GlcNAcβ1→octyl (Lewis X antigen) have been described (21). Galβ1→4GlcNAcβ1→6GalNAcα1→
pura-nitrophenol (core 2 O-glycan) was purchased from
Toronto Research Chemicals (North York, Ontario, Canada).
Fucos was added to extended core 1 O-glycan and to core 2
O-glycan using soluble recombinant α1,2-fucosyltransferase-1
(22). ELISA inhibition assay was performed on SNG cells
grown on 3 mm² glass coverslips (SurgePath, Richmond, IL;
ref. 24). Fixed cells were incubated with HMMC-1 with or
without oligosaccharide inhibitor, followed by fixation with 1% paraformaldehyde in PBS and inactivation of internal peroxi-

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dase with hydrogen oxide in methanol. HMMC-1 bound to
SNG-S cells were then reacted with biotinylated antihuman IgM
antibody and peroxidase-conjugated avidin. Each coverslip was
then incubated in a 96-well plate containing 100 μL peroxidase
substrate ABTS (Pierce), and the absorbance at 405 nm was
recorded by an ELISA reader.

RESULTS

Establishment of the Human Monoclonal Antibody
HMMC-1. KM mice were immunized with SNG-S cells, and
spleen cells were fused with mouse myeloma cells to produce
hybridomas. Approximately 99% of seeded wells produced hy-
bridoma colonies, verifying the efficacy of this method. The
supernatants of these hybridomas were tested for antibodies
reactive to SNG-S cells, which identified 21 positive clones.
Confirmation of these clones was made by immunocytochem-
istry of SNG-S cells and by immunohistochemistry of human
uterine endometrial cancer specimens. The cloned antibody was
designated HMMC-1, for human monoclonal antibody against
müllerian duct-related carcinoma.

Immunohistochemistry performed with the human immu-
noglobulin κ chain as the secondary antibody showed positive
immunostaining, whereas immunostainings using mouse κ
chain as the secondary antibody were negative (data not shown),
indicating that HMMC-1 is a human antibody. The immuno-
globulin subclass was determined by immunoblotting of the
hybridoma supernatant with human IgM and IgG antibodies,
which identified HMMC-1 as a member of the human IgM
subclass (data not shown).

Immunohistochemistry with HMMC-1. HMMC-1 did
not react with normal endometrium and endocervical glands
(Fig. 1, A and B), whereas it reacts with both the luminal surface
and the cytoplasm of adenocarcinoma cells in uterine endome-
trial (Fig. 1C) and endocervical specimens. The luminal surface
of ovarian serous adenocarcinoma and peritoneal serous carcino-
ma was strongly stained with HMMC-1 (Fig. 1, D and H).
Both the luminal surface and cytoplasm were positive in ovarian
mucinous, endometrioid, and clear cell adenocarcinoma (Fig. 1,
E–G). Serous adenocarcinoma of the fallopian tube was weakly
stained with HMMC-1.

As summarized in Table 1, no positive reactivity was
detected with normal endometrium and only slightly with nor-
mal endocervical glands (see Fig. 2 for weak, moderate, and
strong immunostainings). On the other hand, HMMC-1 was
highly reactive with uterine endometrial adenocarcinoma
(54.6%), uterine cervical adenocarcinoma (76.9%), epithelial
ovarian carcinoma (75.0%), and peritoneal serous carcinoma
(87.5%). The positive rates for cervical squamous cell carcino-
ma and for serous adenocarcinoma of the fallopian tube were
relatively low (26.3% and 25%, respectively). The positive rate
for hyperplasia of endometrium was 20.6% and that for atypical
hyperplasia was 31.2%. The histologic grades of endometrial
adenocarcinomas were as follows: 60.9% for well-differentiated
cases, 61.7% for moderately differentiated cases, and 31.8% for
differently differentiated cases. The positive ratings for different
histopathologic types of uterine cervical adenocarcinoma were
85.1% for mucinous adenocarcinoma and 68.0% for endo-
metrioid adenocarcinoma. The positive rate of each histopatho-

Fig. 1 Immunohistochemical staining of uterine endometrial and en-
docervical tissues with HMMC-1. Immunohistochemistry was per-
formed according to protocols listed in the experimental procedures. A,
normal uterine endometrium, proliferative phase; B, normal uterine
docervical glands; C, uterine endometrial adenocarcinoma; D, ovarian
serous adenocarcinoma; E, ovarian mucinous adenocarcinoma; F, ovarian
endometrioid adenocarcinoma; G, ovarian clear cell adenocarcino-
mata; H, peritoneal serous carcinoma (surface serous papillary adeno-
carcinoma). HMMC-1 positivity is not observed in normal endometrium
(A) or normal endocervical glands (B). Cytoplasm and luminal surfaces
are strongly positive in uterine endometrial adenocarcinoma (C). Simi-
larly, the cytoplasm and luminal surface are strongly positive in ovarian
mucinous adenocarcinoma (E), ovarian endometrioid adenocarcinoma
(F), and ovarian clear cell adenocarcinoma (G), whereas the
luminal surface is strongly positive in ovarian serous adenocarcinoma
(D) and peritoneal serous carcinoma (H). Counterstaining was per-
formed with hematoxylin.
logic type of ovarian carcinoma was 75.0% for serous adenocarcinoma, 87.5% for endometrioid adenocarcinoma, 75.0% for mucinous adenocarcinoma, and 62.5% for clear cell adenocarcinoma.

HMMC-1 was faintly reactive with specimens of tissue other than female reproductive organs, namely with epithelium of the collecting duct in kidney and gall bladder specimens (Table 2). However, no staining was seen in normal tissues from stomach, esophagus, colon, liver, pancreas, lung, thyroid gland, testis, or brain specimens, and no staining was detected in cancer specimens from these organs (data not shown).

Table 1: HMMC-1 antibody immunostaining profiles of müllerian-duct related tissues

<table>
<thead>
<tr>
<th>Tissue specimens</th>
<th>Reactivity*</th>
<th>Negative</th>
<th>Weak</th>
<th>Moderate</th>
<th>Strong</th>
<th>Positive rate†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endometrium</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal proliferative endometrium</td>
<td>27</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0% (0/27)</td>
</tr>
<tr>
<td>Normal secretory endometrium</td>
<td>25</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0% (0/25)</td>
</tr>
<tr>
<td>Adenomatous endometrial hyperplasia</td>
<td>23</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>20.6% (6/29)</td>
</tr>
<tr>
<td>Atypical endometrial hyperplasia</td>
<td>11</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>31.2% (5/16)</td>
</tr>
<tr>
<td>Endometrioid adenocarcinoma, G1</td>
<td>16</td>
<td>9</td>
<td>12</td>
<td>4</td>
<td>0</td>
<td>60.9% (25/41)</td>
</tr>
<tr>
<td>Endometrioid adenocarcinoma, G2</td>
<td>13</td>
<td>7</td>
<td>10</td>
<td>4</td>
<td>0</td>
<td>61.7% (21/34)</td>
</tr>
<tr>
<td>Endometrioid adenocarcinoma, G3</td>
<td>15</td>
<td>6</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>31.8% (7/22)</td>
</tr>
<tr>
<td>Cervix</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal cervical gland</td>
<td>22</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8.3% (2/24)</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>14</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>26.3% (5/19)</td>
</tr>
<tr>
<td>Mucinous adenocarcinoma</td>
<td>4</td>
<td>14</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>85.1% (23/27)</td>
</tr>
<tr>
<td>Endometrioid adenocarcinoma</td>
<td>8</td>
<td>13</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>68.0% (17/25)</td>
</tr>
<tr>
<td>Ovary</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serous adenocarcinoma</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>75.0% (6/8)</td>
</tr>
<tr>
<td>Endometrioid adenocarcinoma</td>
<td>1</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>87.5% (7/8)</td>
</tr>
<tr>
<td>Mucinous adenocarcinoma</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>75.0% (6/8)</td>
</tr>
<tr>
<td>Clear cell adenocarcinoma</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>62.5% (5/8)</td>
</tr>
<tr>
<td>Others</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peritoneal serous carcinoma (SSPC)</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>87.5% (7/8)</td>
</tr>
<tr>
<td>Serous adenocarcinoma of the fallopian tube</td>
<td>6</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>25.0% (2/8)</td>
</tr>
</tbody>
</table>

Abbreviation: SSPC, surface serous papillary carcinoma.

*According to the criteria described in Materials and Methods, immunostaining reactivity was classified as negative, weakly, moderately, and strongly positive. Representative images for weakly, moderately, and strongly positive are shown in Fig. 2, A, B, and C, respectively.

†The cases that show more than weakly positive are defined as HMMC-1–positive.

Complement-Mediated Cytolysis by HMMC-1. Complement-mediated cytotoxicity of HMMC-1 was evaluated by in vitro assays. SNG-S cells were reacted with HMMC-1 and then reacted with complement. Lactate dehydrogenase released into the supernatant as a result of cytolysis was monitored. SNG-S cells sensitized with HMMC-1 showed cytolysis, which was dependent on the dose of active complement (Fig. 3). SNG-II cells, which are negative for HMMC-1 antigen, did not show cytolysis by this assay (data not shown). These results indicate that HMMC-1 can kill specifically the target cells through complement-mediated cytolysis.

Characterization of the HMMC-1 Antigen. When an HMMC-1 antigen-positive tissue section was treated with trypsin, tissue specimens showed no changes in reactivity with HMMC-1. By contrast, when sections were treated with periodate, HMMC-1 reactivity was greatly reduced, suggesting that the HMMC-1 antigen is a carbohydrate (data not shown). West-
Western blot analysis of SNG-S cell lysates detected HMMC-1 antigenic bands ranging from 70 to 200 kDa (Fig. 4A, lane 1). When SNG-S cells were cultured in medium containing benzyl N-acetyl α-galactosaminide, an inhibitor of mucin type O-glycan synthesis (16), the levels of HMMC-1 antigen were significantly reduced (Fig. 4A, lanes 2 and 3). These results suggest that HMMC-1 antigen is carried by O-linked carbohydrate.

HMMC-1 antigen expressed on SNG-S cells was completely abolished by benzyl-α-GalNAc (Fig. 4, B and C). This is in contrast to Lewis B antigen, which is partially carried by O-glycan but also carried by glycolipids (13). These results suggest that HMMC-1 determinant is an O-glycan-specific structure.

Identification of Glycosylation Enzymes Required for Synthesis of HMMC-1 Antigen. SNG-S cells strongly express HMMC-1 antigen, whereas the parental line, SNG-II (6, 12), barely expresses HMMC-1 antigen. This observation suggested that SNG-II cells might become HMMC-1 positive if supplemented with one or two glycosyltransferases. As shown in Fig. 5A, panel a, SNG-II cells cotransfected with a mixture of 7 glycosyltransferases (see legend for details) became positive for HMMC-1 antigen, demonstrating that enzymes within this group play a key role in synthesizing the HMMC-1 epitope. When α-1,2 fucosyltransferase-1 was deleted from this mixture, HMMC-1 antigen did not appear in transfected SNG-II cells (Fig. 5A, panel b). Similarly, a mixture of 6 glycosyltransferases without core 1 β1, 3-N-acetylgalactosaminyltransferase could not synthesize HMMC-1 antigen (Fig. 5A, panel c). These results indicate that α1,2-fucosyltransferase-1 and core 1 β1, 3-N-acetylgalactosaminyltransferase are necessary for expression of HMMC-1 antigen. To determine whether these two enzymes are sufficient for HMMC-1 antigen expression, SNG-II cells were cotransfected with α-1,2 fucosyltransferase-1 and core 1 β1, 3-N-acetylgalactosaminyltransferase. As shown in Fig. 5A, panel d, SNG-II cells cotransfected with these two enzymes were strongly positive for HMMC-1, demonstrating that these enzymes are sufficient for SNG-II cells to synthesize the HMMC-1 epitope.

To additionally test sufficiency, COS cells and CHO cells were transfected with a mixture of α-1,2 fucosyltransferase-1 and core 1 β1, 3-N-acetylgalactosaminyltransferase. These cells became positive for HMMC-1 antigen (Fig. 5A, panels e and f). These results strongly suggest that the HMMC-1 epitope is determined by a fucosylated and extended core 1 O-glycan, Fucα1→2Galβ1→4GlcNacβ1→3Galβ1→3GalNAcα1→Ser/Thr.

Effect of Synthetic Oligosaccharides on HMMC-1. To additionally evaluate the specificity of HMMC-1, we chemically synthesized the predicted HMMC-1 antigen oligosaccharide and tested the effect of the oligosaccharide on the reactivity of HMMC-1 to SNG-S cells. An inhibition ELISA assay demonstrated that fucosylated extended core 1 oligosaccharide (Fig. 5B) specifically inhibited binding activity of HMMC-1. This result establishes the epitope of HMMC-1 to be Fucα1→2Galβ1→4GlcNacβ1→3Galβ1→3GalNAcα1→.

### DISCUSSION

The present study describes the production and characterization of a novel human monoclonal antibody, HMMC-1, which recognizes carcinomas of female reproductive organs with high specificity (Figs. 1 and 2; Tables 1 and 2). The in vivo expression pattern of HMMC-1 antigen in cancer cells is closely related to their cell types during embryonic development. Müllerian carcinoma is often used as clinical terminology to denote the broad category of ovarian epithelial car-

<table>
<thead>
<tr>
<th>Normal tissue</th>
<th>Positive/cases examined</th>
<th>Cancerous tissue</th>
<th>Positive/cases examined</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomach</td>
<td>0/6</td>
<td>Stomach</td>
<td>0/6</td>
</tr>
<tr>
<td>Esophagus</td>
<td>0/5</td>
<td>Esophagus</td>
<td>0/5</td>
</tr>
<tr>
<td>Colon</td>
<td>0/6</td>
<td>Colon</td>
<td>0/6</td>
</tr>
<tr>
<td>Liver</td>
<td>0/6</td>
<td>Liver</td>
<td>0/3</td>
</tr>
<tr>
<td>Gall bladder</td>
<td>2/3</td>
<td>Pancreas</td>
<td>0/5</td>
</tr>
<tr>
<td>Pancreas</td>
<td>0/7</td>
<td>Lung</td>
<td>0/7</td>
</tr>
<tr>
<td>Lung</td>
<td>0/9</td>
<td>Kidney</td>
<td>0/5</td>
</tr>
<tr>
<td>Kidney</td>
<td>3/5</td>
<td>Thyroid gland</td>
<td>0/4</td>
</tr>
<tr>
<td>Thyroid gland</td>
<td>0/5</td>
<td>Testis</td>
<td>0/5</td>
</tr>
<tr>
<td>Testis</td>
<td>0/6</td>
<td>Brain</td>
<td>0/2</td>
</tr>
</tbody>
</table>

Table 2 Immunoreactivity of HMMC-1 antibody for various normal and malignant tissues.
cinomas, peritoneal carcinomas, and carcinomas of the fallopian tube, as these malignancies are similar in clinical management. In embryonic origin, however, the uterus and fallopian tube originate from the müllerian duct, which itself is derived from the celomic epithelium. Similarly, the surface epithelium of the ovary also originates from the celomic epithelium. Thus, the HMMC-1 antigen is closely related to a celomic epithelium-derived carcinoma. Therefore, we have assigned the term müllerian-duct related carcinomas to designate both müllerian carcinomas as well as uterine carcinomas, all gynecologic organ carcinomas that express the HMMC-1 antigen.

The present study shows that the HMMC-1 epitope is determined by an O-glycan carbohydrate with a novel structure. The hypothesis that the HMMC-1 epitope is determined by an O-glycan is supported by findings that HMMC-1 antigen is abolished upon culturing SNG-S cells with benzyl-α-GalNAc (Fig. 4), an inhibitor of O-glycan synthesis (16). Many monoclonal anticoagulate antibodies are reactive to the carbohydrate moiety of glycolipid (2). However, it is difficult to determine the carbohydrate epitope if an antibody does not react with the glycolipid. For example, it was not possible to determine the MECA79 antibody epitope until core 2 GnT gene knockout mouse was produced (21). The present study shows the usefulness of transfection with cDNAs encoding glycosyltransferases to elucidate the epitope structure presented by O-glycans.

Although core 1 O-glycan is widely expressed in mammalian epithelial cells, an extended core 1 structure may be rare, as this structure has been reported only in pig oocyte zona pellucida glycoproteins (25, 26), in high endothelial venules (21), and in colon cancer cells (27). Thus, coexpression of α1,2-fucosyltransferase-1 and core 1 α1,3-N-acetylgalactosaminyltransferase in vivo may be limited, leading to an expression pattern of HMMC-1 antigen specific to müllerian duct-related carcinomas.

Human monoclonal antibodies specific to cancer cell surfaces have great therapeutic potential. Thus, the recombinant mouse-human chimeric IgG Trastuzumab increases the clinical benefit of first-line chemotherapy in metastatic breast cancer overexpressing HER2, in addition to its direct actions toward metastatic disease (7, 28). Although our KM mouse produces completely human IgG (11), IgMs produced by our KM mice contain mouse J-chain. In this regard, HMMC-1, which is IgM, is a humanized antibody. Nonetheless, HMMC-1 may be an excellent candidate for cancer therapy, because it is capable of initiating complement-mediated cytotoxicity (Fig. 3). Given that anticoagulate IgM antibody suppresses cancers in vivo (29), HMMC-1 could potentially exhibit anticancer activity in vivo. Due to the i.p. spread demonstrated by most HMMC-1-positive müllerian duct-related carcinomas, HMMC-1 could be particularly effective upon i.p. administration to cancer patients. We are, however, aware of the cautions in predicting clinical application of
IgM antibody. The utility of such antibodies depends in part on the expression pattern of the HMMC-1 antigen in humans, which requires additional analysis. Also, the in vivo activity of HMMC-1 should be determined using tumor-bearing immunodeficient mice. Such analysis will allow us to determine whether this new human monoclonal antibody represents a novel strategy against gynecological malignancies.

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