PRODUCTION AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES TO THE ESTABLISHED HUMAN BLADDER CANCER CELL LINES

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ABSTRACT

In order to identify tumor associated antigen (TAA) on the cell surface of transitional cell carcinoma (TCC) of the bladder, monoclonal antibodies (Mabs) to the established cell line KU-1, derived from transitional cell carcinoma of the bladder, was produced. Cell fusion technique, utilizing spleen cells of immunized Balb/c mouse and SP-2 cells of mouse myeloma cell line, was applied in the present study.

Identification and quantification of cell surface antigen of KU-1 cells and another established cell line derived from bladder carcinoma KU-7 cells were attempted by determining reactivity of the cell surface antigens with thus produced Mabs utilizing enzyme linked immunosorbent assay (ELISA).

Immunohistochemical study demonstrated the cell surface antigen on these two cell lines and surgically removed fresh specimens of TCC of the bladder, using indirect immunofluorescence technique.

Two distinct Mabs (TBSN-1 and TBSN-2) were obtained. TBSN-1 diluted ten fold was observed to be reactive with KU-1 cells but not with KU-7 cells. TBSN-2 diluted twenty fold showed reactivity with KU-7 cells but not with KU-1 cells.

Complement mediated cytotoxicity of these two Mabs against these two cell lines showed higher cytotoxicity of TBSN-1 against KU-7 cells (97.3 ± 15.1% at 1:1 relative concentration of complement) as compared with its cytotoxicity against KU-1 cells (54.2 ± 5.1%). Correlation between complement dose and cytotoxicity of TBSN-1 was statistically significant. TBSN-2 showed markedly higher cytotoxicity against KU-1 cells (57.8 ± 10.5%) than against KU-7 cells (22.2 ± 3.7%), however there was no correlation between complement dose and cytotoxicity of TBSN-2.

Cross reactivity of the Mabs showed higher specificity of TBSN-2 to TCC of the bladder.

The present study disclosed the presence of specific Mab (TBSN-2) to TCC of the bladder. In spite of different histopathological grade of the original tumors, both KU-1 and KU-7 had tumor associated antigen capable of binding with TBSN-2.
Key Words: Monoclonal antibody, Bladder cancer, Established cell line, Tumor associated antigen

INTRODUCTION

Since Köhler and Milstein\(^1\) established a hybridoma secreting Mabs in 1975, the technique has been widely utilized in the fields of biology and medicine. In the field of cancer research, analysis of tumor associated antigens employing Mabs is making rapid progress and some of these antibodies have been utilized for cancer research.\(^2-4\)

There are many evidences to demonstrate the presence of tumor associated antigens of TCC.\(^5,6\) Hashimoto and Masuko\(^7-9\) obtained specific Mab to TCC which had cross reactivity with TCC and epidermal cells, and named corresponding antigen BC-SK antigen. However, Mabs having higher specificity to TCC of the bladder is not currently available.

As an initial step to analyse tumor associated antigen of TCC, an attempt was made to produce Mabs using culture cell line. This study describes production and characterization of the Mabs to the established cell line KU-1 derived from bladder carcinoma.

MATERIALS AND METHODS

1) Cell lines

KU-1 and KU-7 were utilized in the present study. Both cell lines were established at the cell culture laboratory in the Department of Urology, School of Medicine, Keio University. All of these cell lines were grown in monolayer. KU-1, derived from human bladder cancer (TCC Grade 3),\(^10\) was maintained in RPMI-1640 (Gibco, NY, USA) (Fig. 1) and KU-7, established from human bladder cancer (TCC Grade 2),\(^11\) was maintained in Eagle’s minimum essential medium (Chiba Serum, Chiba, Japan) (Fig. 2). Both media were supplemented with 10% fetal bovine serum (FBS; Flow Lab., Virginia, USA) and cell lines were cultured in humidified atmosphere of 5% CO\(_2\), 95% air at 37°C.

SP2/0-Ag14 (SP-2), established from myeloma of Balb/c mouse,\(^12\) was used as parent cells. It was maintained in Dulbeco’s modified minimum essential medium (Flow) with 15% FBS at 37°C in humidified air with 5% CO\(_2\) (Fig. 3).

2) Production of Mabs
A) Immunization

Monolayer of KU-1 cells was trypsinized, harvested and centrifuged at
Fig. 1 Microscopic feature of KU-1 cells is shown (100×). Cells were trigonal in shape and grew flat on the plastic surface.

Fig. 2 Microscopic feature of KU-7 cells is shown (100×). Cells were spindle in shape and rather smaller than KU-1 cells.
Fig. 3 Microscopic feature of SP-2 cells is shown (100×). Cells were round in shape, partly floating in the medium and partly fixed to the plastic surface.

1500 rpm for 5 min. The cells were washed three times and suspended in phosphate buffer solution (PBS). Balb/c mice (4 weeks) were immunized 3 times with peritoneal injection of $10^8$ cells of suspended KU-1 cells per 0.3 ml PBS every two weeks.

B) Cell fusion

Three days after last injection, spleen was removed from immunized mouse. Spleen was floated in medium RPMI-1640 without FBS and torn in small pieces. Single cell suspension of spleen was obtained from the medium after centrifugation and prepared for fusion.

Single cell suspension of SP-2 cells was also prepared. A hundred million spleen cells were mixed with $2\times10^7$ SP-2 cells and centrifuged at 1500 rpm for 5 min. After removal of the medium, mixed cells were fused in 50% polyethylene glycol 4000 (Sigma) using technique as described by Iwasaki. After fusion, the cells were washed once with FBS free RPMI-1640. One million of fused cells in 1 ml of RPMI-1640 with 20% FBS were distributed in 24-well tissue culture plates (Falcon 3047, Becton, Dickinson Lab., Cockeysville, USA). Twenty four hours later, selective medium containing hypoxanthine, aminopterin
Monoclonal Antibodies to Bladder Carcinomas

and thymidine (HAT) (Flow) was added to each well and HAT medium was changed every 2 or 3 days until growth of hybrid colonies was observed.

C) Screening assay of Mabs

Culture medium of each well was analysed by ELISA after hybrid colonies were observed.

KU-1 cells were cultured in 96-well microtitre test plate with V-shaped bottom (Sterilin, Middlesex, England). After emptying the plate, 300 μl blocking solution containing fifteen-fold diluted FBS was added to each well. After removing the solution, 50 μl culture medium of hybridoma was distributed to each well and incubated for 1 hr at room temperature. The plates were then washed by filling each well with 300 μl solution containing 0.2M imidazole buffered with 0.02% Tween 20. Fifty μl peroxidase labeled rabbit anti-mouse IgG+M solution (Kirkegaard & Perry Lab., Meryland, USA) was dropped to each well and incubated for 1 hr at room temperature. Then, 50 μl substrate composed of 2,2'azino-di[3-ethyl-benzthiazoline sulfonate] in cacodylate buffer, pH 5.2, containing 3% hydrogen peroxide (Kirkegaard) was added to each well. The plates were washed 3 times again with Tween 20 solution mentioned above. Positive wells turned blue-green in color.

D) Cloning of hybrid cells

A limiting dilution method was employed. Ninety six-well flat bottomed microtitre plates, which were previously seeded with 100 μl of mouse spleen feeder cells (10⁶/ml in HT medium), were used for growth of hybrid colonies. The hybrid cells of positive wells were diluted to three different concentrations of 6 cells/ml, 60 cells/ml and 600 cells/ml. From these suspension, one hundred μl was added to each well. Each well was checked every day with changing medium every 2 or 3 days.

Culture medium of wells, where monoclonal growth of hybrid cells had been observed, was assayed using ELISA. The cells of positive wells were cloned again.

The hybrid cells producing Mabs in RPMI-1640 with 15% FBS were grown in 25 cm² culture flask (Falcon 3013). Supernatant containing the Mabs was utilized for the following studies.

3) Immunofluorescence staining of KU-1 and KU-7

Monolayer of KU-1 cells or KU-7 cells grown on the Lab Tek chamber (Lab Tek 4802, Illinois, USA) was washed 3 times with PBS and incubated with the obtained Mabs for 1 hr at room temperature. Rabbit FITC labeled anti-mouse IgG + M solution (Kirkegaard & Perry Lab.) was dropped and incubated for 1 hr at room temperature. After gentle washing with PBS, the specimens were observed by a Nikon fluorescence microscope.
4) Cross reactivity of Mabs

Using 9 cell lines and fibroblast, shown in Table 1, cross reactivity of the Mabs was analysed with ELISA. Cross reactivity to surgical specimens taken from bladder, kidney, prostate, liver, gastrointestinal mucosa and skin was also analysed with immunoperoxidase staining or immunofluorescence staining. After taking specimens, frozen sections were made with cryostat and immunoperoxidase or immunofluorescence staining was performed with the Mabs and rabbit FITC labeled anti-mouse IgG + M solution.

Table 1
Cell lines utilized in this study

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Original tumor</th>
<th>Pathology</th>
<th>Culture medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>KU1</td>
<td>Bladder tumor</td>
<td>T.C.C. grade 3</td>
<td>RPMI 1640</td>
</tr>
<tr>
<td>KU7</td>
<td>Bladder tumor</td>
<td>T.C.C. grade 2</td>
<td>Eagle's MEM</td>
</tr>
<tr>
<td>NBT</td>
<td>Bladder tumor</td>
<td>T.C.C. grade 4</td>
<td>RPMI 1640</td>
</tr>
<tr>
<td>T24</td>
<td>Bladder tumor</td>
<td>T.C.C. grade 3</td>
<td>Eagle's MEM</td>
</tr>
<tr>
<td>MGH-U1</td>
<td>Bladder tumor</td>
<td>T.C.C. grade 4</td>
<td>Eagle's MEM</td>
</tr>
<tr>
<td>DU145</td>
<td>Prostatic carcinoma</td>
<td>Adenocarcinoma</td>
<td>Eagle’s MEM</td>
</tr>
<tr>
<td>PC3</td>
<td>Prostatic carcinoma</td>
<td>Adenocarcinoma</td>
<td>Eagle’s MEM</td>
</tr>
<tr>
<td>KU2</td>
<td>Renal cell carcinoma</td>
<td>Clear cell carcinoma</td>
<td>Eagle’s MEM</td>
</tr>
<tr>
<td>HeLa</td>
<td>Uterus carcinoma</td>
<td>Adenocarcinoma</td>
<td>Eagle’s MEM</td>
</tr>
<tr>
<td>SP2</td>
<td>Balb/c myeloma</td>
<td>(−)</td>
<td>Dulbecco's modified MEM</td>
</tr>
</tbody>
</table>

T.C.C.: transitional cell carcinoma.
MEM: minimum essential medium.

5) Reactivity of Mabs to KU-1 and KU-7

Culture medium containing Mabs produced by \(2 \times 10^6\) hybrid cells after 3 days incubation was prepared. KU-1 and KU-7 were grown in microtitre plate to be studied with ELISA. Culture medium with Mabs with 7 different concentrations, \(1:1, 1:2, 1:5, 1:10, 1:20, 1:50\) and \(1:100\) were tested for reactivity against KU-1 and KU-7.

6) Characterization of Immunoglobulins (Ig)

Ig class and subclass of Mabs with rabbit antisera to mouse Ig (anti-IgM; Zymed Lab., California, USA. anti-IgG1, G2, G3; Nordic Immunology, Tilburg, Netherlands) were examined according to technique of ELISA as described above with some modifications. After reacting KU-1 with Mabs and rabbit anti-mouse Ig serum and washing the plate, goat peroxidase labeled anti-rabbit Ig serum (Cappel, PA, USA) was added to each well and incubated for 1 hr at room
temperature. After washing 5 times with washing solution, substrate solution was dropped to wells, judging the positive well after 30 min.

7) Complement mediated cytotoxicity test

Two million hybrid cells were cultured in 5 ml RPMI-1640 with 15% inactivated FBS for 3 days. The culture medium was assayed in this test. A million of KU-1 cells and KU-7 cells were incubated in 1 ml RPMI-1640 with 50 μCi \(^{51}\)Cr \((\text{Na}_2^{51}\text{CrO}_4)\). \(^{51}\)Cr-labeled KU-1 and KU-7 were dispersed into 96-well microtitre plate \((10^4 \text{ cells/well})\). Two hundred μl culture medium of hybrid cells was added to each well with appropriately diluted normal rabbit serum \((\text{Cedarlane Lab., Ontario, Canada})\) as a source of complement. Complement was diluted to 7 different concentrations, complement free, 1:1, 1:5, 1:10, 1:50, 1:100 and 1:500. After 4 hrs incubation in 5% CO\(_2\) with 95% air at 37°C, \(^{51}\)Cr release was counted by γ-counter. As a control study, cytolysis of RPMI-1640 with 15% FBS \((\text{medium NS-1})\) and without FBS was also tested. Percent cytotoxicity was calculated with a formula mentioned below,

\[
\text{% cytotoxicity} = \frac{\text{release by Mab & C. - spontaneous release in presence of C.}}{\text{complete release by minimum release}} = \frac{\text{2\% Triton X}}{\text{(C. free medium)}}\]

RESULTS

1) Production of Mabs

Seven days after fusion of SP-2 cells and spleen cells, colonies of hybrid cells were observed in 90% of 24-well culture plate \((\text{Fig. 4})\). Since the cells had different growth rates, hybrid cells which had grown early, were subcultured to different culture plates. Two weeks after fusion, supernatants were assayed to see whether the hybridoma produced Mabs with the use of ELISA staining. Four of 64 wells were positive \((\text{Fig. 5})\). Each positive well had \(1 \times 10^5 \text{ to } 5 \times 10^5\) hybrid cells, and cloning with limiting dilution method was performed after screening assay. Cloning rate was shown in Table 2. After cloning, two Mabs \((\text{TBSN-KU-1-1 [TBSN-1], and TBSN-KU-1-2 [TBSN-2]})\) were obtained. Culture medium containing Mabs was frozen and hybrid cells were also stocked in liquid nitrogen.

2) Immunohistochemical studies

KU-1 cells, KU-7 cells in monolayer and frozen sections of bladder carcinomas were stained by indirect immunofluorescence technique with TBSN-2 \((\text{Fig. 6})\). Some of cells showed strong fluorescence.
Fig. 4-a) Microscopic appearance of hybrid colony which is observed 7 days after cell fusion, was shown (100×). Cells around the colony were devitalized with the selective medium HAT.

Fig. 4-b) Microscopic feature of hybrid colony which was observed 14 days after cell fusion, is shown (100×). The colony occupied almost whole surface of the well.

Fig. 5 Screening assay of culture media in which hybrid colonies were observed. A) Wells of positive control changed bluegreen in color. B), C), D) and E) Positive wells were identified.
Table 2
Cloning rate

<table>
<thead>
<tr>
<th>Seeding cell/well</th>
<th>0.6</th>
<th>6</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monoclone wells</td>
<td>4.7±2.1/96</td>
<td>16.7±1.2/96</td>
<td>14.3±5.7/96</td>
</tr>
<tr>
<td>Polyclone wells</td>
<td>0/96</td>
<td>2.6±2.3/96</td>
<td>6.7±2.1/96</td>
</tr>
</tbody>
</table>

3) Specific immunoreactivity of Mabs

Cross reactivity of Mabs was analysed using cell line panel. TBSN-1 had cross reactivity to KU-7, T-24, PC-3, DU-145 and HeLa. TBSN-2 had reacted to KU-7 and HeLa. Both Mabs had no binding activity to NBT, MGH-U1 and fibroblast (Table 3, Fig. 7).

TBSN-2 reacted strongly with frozen section of bladder carcinomas, while there was no demonstrable reactivity of TBSN-1 to these tissue specimens. The binding specificity of TBSN-2 to bladder carcinomas was confirmed by the facts that TBSN-2 as well as TBSN-1 could neither bind with normal bladder epithelium, nor with the other organs (Table 4).
Table 3
Cross reactivity of Mabs to cell line panel

<table>
<thead>
<tr>
<th></th>
<th>TBSN-1</th>
<th>TBSN-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>KU1</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>KU7</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>NBT</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>T24</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>MGH-U1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Du145</td>
<td>±</td>
<td>-</td>
</tr>
<tr>
<td>Pc3</td>
<td>±</td>
<td>-</td>
</tr>
<tr>
<td>KU2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HeLa</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Fibroblast</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Fig. 7 Cross reactivity of TBSN-1 and TBSN-2 against cell line panel was analysed with duplicated study of ELISA. A) KU-1, B) KU-7, C) NBT, D) T-24, E) MGH-U1, F) DU-145, G) PC-3, H) KU-2, I) HeLa and J) Fibroblast.

3) Detection of Ig class and subclass

Ig class and subclass of Mabs were analysed by ELISA using class and subclass specific rabbit anti-mouse Ig sera. TBSN-1 was determined as IgG2 and TBSN-2 as IgM (Table 5, Fig. 8).
### Table 4
*Cross reactivity to surgical specimens*

<table>
<thead>
<tr>
<th>Surgical specimens</th>
<th>Staining method</th>
<th>TBSN-1</th>
<th>TBSN-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bladder cancer</td>
<td>IF</td>
<td>(−)</td>
<td>(+)</td>
</tr>
<tr>
<td>Normal bladder epithelium</td>
<td>IF</td>
<td>(−)</td>
<td>(−)</td>
</tr>
<tr>
<td>Liver</td>
<td>IP</td>
<td>(−)</td>
<td>(−)</td>
</tr>
<tr>
<td>Kidney</td>
<td>IP</td>
<td>(−)</td>
<td>(−)</td>
</tr>
<tr>
<td>Prostate</td>
<td>IP</td>
<td>(−)</td>
<td>(−)</td>
</tr>
<tr>
<td>Gl mucosa</td>
<td>IP</td>
<td>(−)</td>
<td>(−)</td>
</tr>
<tr>
<td>Skin</td>
<td>IP</td>
<td>(−)</td>
<td>(−)</td>
</tr>
</tbody>
</table>

*IF: immunofluorescence, IP: immunoperoxidase*

### Table 5
*Class and subclass*

<table>
<thead>
<tr>
<th></th>
<th>TBSN-1</th>
<th>TBSN-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti Ig M</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Anti Ig G₁</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Anti Ig G₂</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Anti Ig G₃</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

Fig. 8 Identification of Ig class and subclass was performed with rabbit antimouse sera. TBSN-1 was detected as IgG₂ whereas TBSN-2 as IgM.
4) Binding activity of diluted Mabs to KU-1 and KU-7

Both two Mabs had cross reactivity to KU-1 and KU-7. TBSN-1 had a strong binding activity to KU-1 than to KU-7. TBSN-1 at 1:10 dilution was still able to bind with KU-1, but KU-7 could be bound with TBSN-1 at 1:2 dilution. On the other hand, TBSN-2 had a stronger reactivity to KU-7 than to KU-1, however, at 1:10 dilution failed to show reactivity with KU-1. Both

<table>
<thead>
<tr>
<th></th>
<th>TBSN-1</th>
<th>TBSN-2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>KU-1</td>
<td>KU-7</td>
</tr>
<tr>
<td>1:1</td>
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<td>+</td>
</tr>
<tr>
<td>1:2</td>
<td>+</td>
<td>±</td>
</tr>
<tr>
<td>1:5</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>1:10</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>1:20</td>
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<td>−</td>
</tr>
<tr>
<td>1:50</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>1:100</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

Fig. 9 Binding reactivity of diluted Mabs with KU-1 and KU-7 was examined with ELISA. TBSN-1 had a stronger reactivity with KU-1 than KU-7, whereas TBSN-2 had a stronger reactivity with KU-7 than KU-1. (C: negative control)
Mabs at 1:50 and 1:100 dilution were negative in ELISA reaction (Table 6, Fig. 9).

5) Cytotoxic activity with complement

Both culture supernatant from NS-1 and FBS free medium RPMI-1640 as the control media had no significant cytotoxic activity to neither KU-1 nor

<table>
<thead>
<tr>
<th>Cell line complement concentration</th>
<th>NS-1</th>
<th>RPMI 1640</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>KU-1</td>
<td>KU-7</td>
</tr>
<tr>
<td>(-)</td>
<td>674.7 ± 5.0</td>
<td>153.7 ± 8.0</td>
</tr>
<tr>
<td>1:1</td>
<td>704.7 ± 5.0</td>
<td>174.6 ± 16.0</td>
</tr>
<tr>
<td>1:5</td>
<td>672.3 ± 11.6</td>
<td>145.0 ± 12.1</td>
</tr>
<tr>
<td>1:10</td>
<td>675.0 ± 16.1</td>
<td>165.7 ± 15.0</td>
</tr>
<tr>
<td>1:50</td>
<td>690.7 ± 24.0</td>
<td>166.0 ± 16.5</td>
</tr>
<tr>
<td>1:100</td>
<td>684.3 ± 12.3</td>
<td>157.3 ± 19.0</td>
</tr>
<tr>
<td>1:500</td>
<td>681.3 ± 12.3</td>
<td>150.3 ± 15.6</td>
</tr>
<tr>
<td>MR</td>
<td>2707.5 ± 135.1</td>
<td>776.3 ± 14.6</td>
</tr>
</tbody>
</table>

Control study of cytolysis with complement alone and without complement

(mean ± SD: cpm)

NS-1: RPMI 1640 with 15% FBS, MR: maximum release with Triton X

TBSN-1

Fig. 10 Percent cytotoxicity of TBSN-1 with complement is shown. Higher cytotoxicity against KU-7 than KU-1 was observed.
KU-7, except that they had approximately 1 to 3% cytotoxicity with high concentration of complement at 1:1 dilution (Table 7). Under presence of Mabs, complement mediated cytotoxicity was remarkable. TBSN-1 had significantly greater activity of complement mediated cytotoxicity against KU-7 (97.3 ± 15.1% at 1:1 dilution of complement) as comparing cytotoxicity against KU-1 (54.2 ± 5.1%) (p<0.01). Cytotoxicity against KU-7 (22.6 ± 1.8%) was found at 1:500 dilution. There was a statistically significant positive correlation between complement dose and cytotoxicity generated by TBSN-1 (p<0.05) (Fig. 10). TBSN-2 had also cytotoxic activity in the presence of complement to KU-1 and KU-7. Cytotoxic activity against KU-1 (57.8 ± 10.5% at 1:1 concentration) was also significantly higher than that against KU-7 (22.3 ± 3.7%) (p<0.05). However, there was no correlation between complement dose and cytotoxicity generated by TBSN-2 (Fig. 11).

DISCUSSION

KU-1, the established cell line, was used as a source of antigen to analyse TAA of the bladder cancer in this study. Two distinct Mabs were obtained by cell fusion technique. Old14 classified cell surface antigens to three categories: Class 1 antigens are tumor specific antigens, which are not demonstrable on any other normal or malignant cells, Class 2 antigens are shared tumor antigens found on autologous as well as on allogenic tumors of similar and dissimilar
origin and Class 3 antigens are widely distributed on normal and malignant
cells, both autologous and xenogenic. Serological and immunological analyse of
TBSN-1 and TBSN-2 showed that corresponding antigen with TBSN-1 was a
class 2 antigen, whereas corresponding antigen with TBSN-2 was a class 1
antigen, and that these Mabs recognized different antigenic determinants which
were demonstrated on the cell surfaces of both KU-1 and KU-7.

Therefore the corresponding antigen with TBSN-2, thought to belong to
TAA of TCC is shown to have higher specificity to TCC than BC-SK antigen
reported to be demonstrable not only on TCC but also on the skin from Hashi-
moto and Masuko.7-9 Thus, BC-SK antigen was not strictly considered to belong
to class 1 antigen.

Cell lines utilized in this study were all human cancer cell lines. Thus,
these cell lines might have human major histocompatibility antigens including
HLA antigens, in particular. Parmiani et al.15 described that tumor cells had a
possibility to obtain new and foreign histocompatibility antigen on the tumor
cells, and that the expression of histocompatibility antigen on tumor cells might
be altered. Both TBSN-1 and TBSN-2 belonged to mouse immunoglobulin be-
cause SP-2 was derived from mouse myeloma. Because of no human myeloma
cell lines available for production of Mabs, SP-2, reported to be a preferable
cell line as parent cells by Shulman,12 was utilized in this study. Therefore
possibility for TBSN-1 and TBSN-2 to bind with HLA antigens remains to be
undermined. The technique (Microdroplet test) introduced by Terasaki16 is
available to detect HLA antigens of human lymphocytes. However, the tech-
nique could not be applied to identify HLA antigens on KU-1 and KU-7, because
there was a possibility of nonspecific staining of KU-1 and KU-7 cells with
trypan blue which was utilized for judgement of this test. Despite some like-
liness of the Mabs binding with HLA antigens, both TBSN-1 and TBSN-2
were clearly shown to have cross reactivity with various cell lines which of
totally unrelated origins. Therefore it is conceivable that KU-1 and KU-7 do
have two shared antigens distinguishable from HLA antigen, indicating the
presence of TAAs despite difference in histopathological grade of original
bladder carcinomas. Fazio and Gozzo17 also reported that they could detect TAA
of TCC in the urine of patients with different grade and stage.

KU-1 and KU-7 were shown to share at least two antigens, however, the
amount of the antigens on the cell surface of each cell line was different as
shown by the results of reactivity of the Mabs with KU-1 and KU-7 (Table 6),
and this indicated that the amount of the antigen bound with TBSN-1 per cell
of KU-1 was more than that of KU-7, whereas the amount of the antigen bound
with TBSN-2 per cell of KU-7 was more than that of KU-1. It is conceivable,
however, that the amount of the antigen per cell of each cell line could vary
because of heterogenous populations constituting each cell line. In pursuit of the utility of the Mabs, characterization of the Mabs was attempted by analysing classes and subclasses of immunoglobulins and determining complement mediated cytotoxicity. For complement mediated cytotoxicity, rabbit fresh serum was used because of higher complement activity of rabbit serum than that of human serum. It is known that one molecule of pentameric IgM can activate the complement pathway, whereas at best two adjacent molecules of IgG appear to be required. Thus, complement mediated cytotoxicity is considered to depend on distribution of antigens on the cell surface. Complement mediated cytotoxicity serving as control when either RPMI-1640 without FBS or RPMI-1640 with 15% FBS (NS-1) was used showed low cytotoxicity (approximately 1 to 3%) in the presence of high concentration of complement. The observed cytolysis was thought to be caused by a mechanism of direct cytolytic function of complement as proposed by Götze and Thompson.

The cytotoxicity test of this study showed TBSN-1 to belong to IgG2 and have higher cytolytic activity against KU-7 than KU-1, whereas TBSN-2 to IgM and higher cytolytic activity against KU-1 than KU-7. These findings suggested that the antigens bound with TBSN-1 were distributed evenly on the surface of all KU-7 cells regardless of its subpopulations, however unevenly on the cell surface of KU-1 cells. It is also suggested that the antigens bound with TBSN-2 were homogeneously distributed on the surface of KU-1 cells but not homogeneously on the surface of KU-7 cells.

Based on these findings, each of these two cell lines (KU-1 and KU-7) consists of polyclonal populations of cells having different distribution and amount of the antigens. Despite differences in reactivity of TBSN-2 with each of these two cell lines, usefulness of TBSN-2 was strongly suggested to increase our understanding about TAA of TCC of the bladder.

CONCLUSIONS

1) Specific Mab (TBSN-2) to transitional cell carcinoma of the bladder was obtained.
2) Tumor associated antigen could be demonstrated on the cell surface of transitional cell carcinoma regardless of histopathological grade.
3) KU-1 and KU-7 had two common antigens. One was tumor associated antigen, however the other antigen was demonstrated on the cell surface of another cancer cell lines.
4) Each cell line had different distribution and amount of these antigens on the cell surface.
5) According to high specificity of TBSN-2, it is considered to increase our understanding of tumor associated antigen of the bladder cancer.

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