Malignant Lymphoma Antigen Expressed in Nude Mouse Tumor Cells Derived from Carcinogen-transformed Bloom’s Syndrome B-Lymphoblastoid Cell Lines

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ABSTRACT

From nude mouse tumors, in which malignant transformed Bloom’s syndrome (BS) B-lymphoblastoid cell lines were successfully transplanted into s.c. tissues, we have detected strong expression of malignant lymphoma (ML)-associated antigen on the cell surface, by using diluted sera of ML patients and indirect immunofluorescence. Even though carcinogen-treated BS B-lymphoblastoid cell lines expressed various types of cancer antigens (ML, ovarian cancer, stomach cancer, lung cancer, liver cancer, etc.) on the cell membrane as a mixed population (Y. Shiraishi and H. Soma, Proc. Natl. Acad. Sci. USA, 85: 8211–8215, 1988), the finding that BS malignant cells originating from nude mouse tumors expressed specific ML-associated antigen seemed significant for ML diagnosis. BS nude mouse tumors were successfully transplanted from nude mice to nude mice (100%). Histopathological studies using an electron microscope demonstrated that most tumor cells in s.c. tissues of nude mice were lymphoid malignant cells. Gel electrophoresis and Western blotting analyses demonstrated that the antigen which characterized ML was a single band (M, 97,000) and did not cross-react with the sera of other cancer patients or normal sera. Chromosome analysis showed that the cell clones with ML-associated antigen had marker chromosomes involving (t(6;7)(p25;q27), t(9;7)(q34;7), del(10)(p13), t(12;14)(q24;q11)). The expression of ML-associated antigen was also discussed in relation to the marker chromosomes.

INTRODUCTION

Since our previous studies (1–3) have shown that BS B-LCLs are highly susceptible to malignant transformation following carcinogen treatments, as evidenced by tumor production in nude mice, and since BS patients are highly predisposed to various malignant diseases, we have been interested whether the BS nude mouse tumor is at all malignant in view of its related diseases on cell membranes, although the other nude mouse tumors expressed specific cancer antigens of ML patients and indirect immunofluorescence. Kven though the BS nude mouse tumor is at all malignant in view of its related diseases (CANCER RESEARCH 50. 3106–3112. May 15, 1990), investigations revealed that malignantly transformed BS B-LCLs expressed various types of cancer antigens (ML, ovarian cancer, stomach cancer, lung cancer, liver cancer, etc.) on the cell membrane as a mixed population (4). We have examined BS nude mouse tumors histologically and ultrastructurally and have tested the ML antigen (BS-derived tumor cells) in the reaction with the antibody of patients’ sera (ML and related diseases) by use of an indirect immunofluorescence technique. To further evaluate the character of this antigen, we analyzed the membrane antigen protein from BS-derived ML antigens with the use of WB analysis and identified the molecular weight of the antigen. We have also analyzed karyotypes in malignant cell clones with ML-associated antigen and discussed the marker chromosomes relating to the expression of ML-associated antigen.

MATERIALS AND METHODS

Cell Lines and Cell Culture. A permanent Bloom’s syndrome B-lymphoblastoid cell line (B-LCL) (BS-SY) was established from a patient with Bloom’s syndrome (1, 2, 5, 6). As described previously, this cell line was found to have characteristicly increased SCE levels [average SCE value, 71.6 ± 2.77 (SD)] in cells labeled with bromodeoxyuridine for two cell cycles. Even though chromosome instability, including breaks (6%) and quadriradials (0.02%), was detected at relatively low frequency, the karyotype of these cells was basically normal (46,XY in BS-SY). The cells were maintained in RPMI 1640 supplemented with 10% fetal calf serum at 37°C in a 5% CO2/95% air humidified incubator. In all cell lines established with Epstein-Barr virus, tests of cell surface markers, such as clumping of lymphoid cells, negative spontaneous rosette formation, and positive surface immunoglobulins, revealed the lymphocytes to be B-cell origin (7, 8).

Malignant Transformation with Carcinogens. Bloom’s syndrome (BS-SY) and normal (KS6) (1, 2, 5, 6) cells (2 x 107) were exposed to MNNG and/or 4NQO at 0.4 µg/ml for 24 h, washed with fresh RPMI 1640, and then reincubated in RPMI 1640 supplemented with 10% fetal calf serum at 37°C in a 5% CO2/95% air incubator for 20–30 days. To examine the tumorigenicity, the carcinogen-treated BS B-LCLs (1 x 107 cells) were inoculated into the s.c. tissue of 6-week-old nude mice (BALB/c-nu/nu). Tumor appeared at 3–4 weeks in the s.c. portion which was inoculated (Fig. 1). Since tumors were successfully transplanted into nude mice at third passages (Fig. 2), s.c. tumors were removed, dissected, and transferred to in vitro culture again. MNNG-treated BS-SY-derived tumor (designated as BS-SY; TM) proliferated actively in vitro and showed strong antigen associated with ML and related diseases on cell membranes, although the other nude mouse tumors, which originated from different cell clones and was also retransplantable, were not possible to be recultured in vitro. Therefore, BS-SY, TM cells were used as an antigen for the immunofluorescent procedure in the reaction with patients’ sera. From recultured tumor cells, surface markers (immunoglobulin, Leu-4, Leu-12) and the karyotype were examined and antigen characters were evaluated.

Histopathological Study. Tumors s.c. (1.7 x 2.5 cm), axillary and inguinal lymph nodes, spleens, livers, lungs, and kidneys were also processed for histological studies. The s.c. tumors were cut and divided into two parts for histological and ultrastructural studies. One portion was fixed in 10% buffered neutral formalin and embedded in paraffin. Sections 3 µm thick were stained with hematoxylin and eosin, Giemsa, methyl green-pyronin, and reticulin. Other organs and tissues obtained from nude mice were routinely stained with hematoxylin and eosin.

For ultrastructural study, tumor tissues were fixed in 2.5% phosphate-buffered glutaraldehyde at 4°C for 2 h at pH 7.2, then washed 3 times in phosphate buffer, and postfixed in 1% phosphate-buffered osmium tetroxide for 1 h at 4°C at pH 7.2. After dehydration in ascending concentrations of ethanol, the specimens were embedded in Epon. Sections 1 µm thick were stained with Richardson’s solution and examined by light microscopy. Ultrathin sections cut with a Reichert Jung OmU4 ultramicrotome were stained with uranyl acetate and lead citrate before examination with a Hitachi H-800 electron microscope.
was taken 45 days after the inoculation. Cells were then washed with cold PBS twice, reacted twice, were suspended in 1:30 PBS diluted sera (300 μl) of patients with ML and related diseases (Table 3) at 4°C for 90 min for antigen-detection of cell surface antigens, cells (3 x 10⁶), washed with PBS, and overlapped with nitrocellulose membrane (ADVANTEC; Toyo) for blotting. Western blots were performed by standard methods as described previously (14). The avidin-biotin immunoperoxidase procedure for detecting ML antigen using antibodies of patients’ sera was carried out as described (15, 16). Transferred nitrocellulose membranes (ML antigens) were treated with 300 μl of ML patients’ sera (or other sera) and mixed approximately 1:1, v/v (1 x 10⁷-1 x 10⁸ cells/ml serum) with antigen cells. BS-SY, TM cells were washed three times with PBS and sealed with nonimmunofluorescent glycerin. Absorptions were carried out for l h at 4°C and then l h at 37°C. Specimens were centrifuged at 2000 rpm for 20 min at 4°C and then 1 h at 37°C. Specimens were centrifuged at 1000 rpm for 5 min at 0°C to remove nucleus, and concentrated three times with Amicon B15 membrane filter. ML antigen protein samples (20 μg), prepared by heating at 100°C for 3 min in the sample buffer (containing 5% 2-mercaptoethanol and 2% SDS), were placed on the top of the 4–20% gradient gel and SDS-polyacrylamide gel electrophoresis was performed according to the procedure described by Laemmli (13). After electrophoresis, the gel was washed in Tris buffer (pH 7.2) to remove SDS and overlapped with nitrocellulose membrane (ADVANTEC; Toyo) for blotting. Western blots were performed by standard methods as described previously (14). The avidin-biotin immunoperoxidase procedure for detecting ML antigen using antibodies of patients’ sera was carried out as described (15, 16). Transferred nitrocellulose membranes (ML antigens) were treated with 300 μl of ML patients’ sera (or other sera) and mixed approximately 1:1, v/v (1 x 10⁷-1 x 10⁸ cells/ml serum) with antigen cells. BS-SY, TM cells were washed three times with PBS and mixed approximately 1:1, v/v (1 x 10⁷-1 x 10⁸ cells/ml serum) with the serum to be tested. Absorptions were carried out for 1 h at 4°C and then 1 h at 37°C. Specimens were centrifuged at 2000 rpm for 20 min at 4°C. Absorbed serum was tested, together with an aliquot of the same dilution of unabsorbed serum as for both membrane immunofluorescence and WB.

**RESULTS**

**Malignant Transformation and Transplantation into Nude Mice**

Characterization of the B-LCLs established from a normal individual (KS86) and a BS patient (BS-SY, ) before and after carcinogen treatment are shown in Table 1. BS-SY, cells was inoculated of malignantly transformed BS cells into s.c. tissues of nude mice. For the detection of cell surface antigens, cells (3 x 10⁶), washed with PBS twice, were suspended in 1:30 PBS diluted sera (300 μl) of patients with ML and related diseases (Table 3) at 4°C for 90 min for antigen-antibody reaction. Cells were then washed with cold PBS twice, reacted with fluorescein isothiocyanate-conjugated goat anti-BSY, TM (cultured) cells to test whether serum antibody reacted specifically with human IgG (1:40 dilution with PBS) at 4°C for 90 min, washed with cold PBS twice, and sealed with nonimmunofluorescent glycerin and a cover slip. Immunofluorescence (IF) was determined with an Olympus fluorescence microscope with epiillumination.

**Table 1 Characterization of B-lymphoblastoid cell lines from normal (KS86) and Bloom’s syndrome (BS-SY,) blood samples following carcinogen treatments**

<table>
<thead>
<tr>
<th>Carcinogen treatment, 0.4 μg/ml, 24 h</th>
<th>Surface immunoglobulin (%)</th>
<th>SCE level (mean ± SE)</th>
<th>Karyotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell lines</td>
<td>α γ µ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KS86</td>
<td>0 21* 0</td>
<td>4.8 ± 0.03</td>
<td>46,XY</td>
</tr>
<tr>
<td>4NQO</td>
<td>0 19 0</td>
<td>5.1 ± 0.03</td>
<td>46,XY</td>
</tr>
<tr>
<td>MNNG</td>
<td>0 17 0</td>
<td>5.2 ± 0.04</td>
<td>46,XY</td>
</tr>
<tr>
<td>BS-SY,</td>
<td>0 0 14</td>
<td>71.6 ± 2.77</td>
<td>46,XY</td>
</tr>
<tr>
<td>4NQO</td>
<td>0 0 16</td>
<td>72.4 ± 3.10</td>
<td>46,XY/abnormal clones</td>
</tr>
<tr>
<td>MNNG</td>
<td>0 0 18</td>
<td>70.9 ± 2.99</td>
<td>46,XY/abnormal clones</td>
</tr>
</tbody>
</table>

* Average values of >500 cells in each entry.

These data were examined in KS86 and BS-SY, cells cultured for about 3 weeks following 24 h carcinogen treatment. The picture was taken 45 days after the inoculation.

Fig. 1. Nude mouse bearing a solid tumor of BS-SY, TM cells. The picture was taken 45 days after the inoculation.

Fig. 2. Schematic representation of carcinogen treatments for BS B-LCLs and inoculations of malignantly transformed BS cells into s.c. tissues of nude mice.

Millonig’s lead and examined with a JEM 100S type electron microscope.

Detection of Membrane Antigens Associated with Cancer. To examine the cancer antigen character in the recultured tumor cells, cells (BS-SY, TM) that were suspended by pipeting were washed twice in PBS. Cell surface antigens were tested using fresh cell suspension by an indirect immunofluorescence with fluorescein isothiocyanate-conjugated goat anti-sera monospecific for human IgG (4, 9–12). For the detection of cell surface antigens, cells (3 x 10⁶), washed with PBS twice, were suspended in 1:30 PBS diluted sera (300 μl) of patients with ML and related diseases (Table 3) at 4°C for 90 min for antigen-antibody reaction. Cells were then washed with cold PBS twice, reacted with fluorescein isothiocyanate-conjugated goat anti-BSY, TM (I) cells, were dissolved in 4.0 ml of Tris-Triton buffer (10 mM Tris-HCl, pH 7.4—150 mM NaCl, 0.5% Triton X-100—0.2 mM phenylmethylsulfonyl fluoride), centrifuged at 10000 rpm for 5 min at 0°C to remove nucleus, and concentrated three times with Amicon B15 membrane filter. ML antigen protein samples (20 μg), prepared by heating at 100°C for 3 min in the sample buffer (containing 5% 2-mercaptoethanol and 2% SDS), were placed on the top of the 4–20% gradient gel and SDS-polyacrylamide gel electrophoresis was performed according to the procedure described by Laemmli (13). After electrophoresis, the gel was washed in Tris buffer (pH 7.2) to remove SDS and overlapped with nitrocellulose membrane (ADVANTEC; Toyo) for blotting. Western blots were performed by standard methods as described previously (14). The avidin-biotin immunoperoxidase procedure for detecting ML antigen using antibodies of patients’ sera was carried out as described (15, 16). Transferred nitrocellulose membranes (ML antigens) were treated with 300 μl of ML patients’ sera (or other sera) and mixed approximately 1:1, v/v (1 x 10⁷-1 x 10⁸ cells/ml serum) with the serum to be tested. Absorptions were carried out for 1 h at 4°C and then 1 h at 37°C. Specimens were centrifuged at 2000 rpm for 20 min at 4°C. Absorbed serum was tested, together with an aliquot of the same dilution of unabsorbed serum as for both membrane immunofluorescence and WB.

**Chromosome Analyses of Tumors.** Chromosome spreads were prepared by a routine air-drying method (17) from tumor cells which appeared in the s.c. tissue of nude mice and from recultured tumor cells in vitro. Differential staining of sister chromatids and karyotype analysis were performed by using the fluorescence plus Giemsa technique (18–20) and G- and Q-banding, respectively. For SCE analysis, the cells were labeled with bromodeoxyuridine at 10 μg/ml for 48 h.

**RESULTS**

**Malignant Transformation and Transplantation into Nude Mice**

Characterization of the B-LCLs established from a normal individual (KS86) and a BS patient (BS-SY, ) before and after carcinogen treatment are shown in Table 1. BS-SY, cells was
TABLE 2  Tumorigenicity, cytogenetic, and immunological characterization of tumor cells in nude mice

<table>
<thead>
<tr>
<th>Cell origin</th>
<th>Treatment, 0.4 µg/ml, 24 h</th>
<th>Surface markers</th>
<th>Tumor</th>
<th>S Ig*</th>
<th>Leu-4</th>
<th>Leu-12</th>
<th>EBNA</th>
<th>Karyotype</th>
</tr>
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<tbody>
<tr>
<td>KS86</td>
<td>None (0/6)*</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
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<tr>
<td></td>
<td>MNNG (0/7)</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>4NQO (0/8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BS-SY,</td>
<td>None (0/6)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>4NQO (3/7)</td>
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<tr>
<td></td>
<td>MNNG (5/7)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>BS-SY, TM I</td>
<td>8-I</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>BS-SY, TM II</td>
<td>8-II</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>BS-SY, TM III</td>
<td>8-III</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

* S Ig, surface immunoglobulin; EBNA, Epstein-Barr nuclear antigen; NT, not tested.
* Numbers in parentheses, number of nude mice with tumors/number of inoculated nude mice.

IgM producers with cytoplasmic and surface \( \mu \) and light chains, whereas KS86 cells were IgG positive. Normal cells treated with MNNG or 4NQO showed no changes in the cell surface or cytoplasmic immunoglobulins or in the karyotypes. These cells did not produce tumors in nude mice. The frequency of IgM-positive cells in carcinogen-treated BS-SY, decreased to one-half of the level, compared to nontreated conditions (Table 1).

When the carcinogen-treated BS-SY, cells were cultured in vitro for 20–30 days and inoculated s.c. into nude mice, tumors appeared at an average rate of 50–60% (Table 2). When tumors that originated from MNNG-treated BS-SY, cells were retransplanted, they grew well and were carried successfully for two to three passages from one nude mouse to the next until discontinued (100% transplantable). This strongly supports the notion that the Bloom syndrome tumors are malignant. Surface markers (immunoglobulin, Leu-4, and Leu-12) were not detectable in any of the tumors, but they were Epstein-Barr virus nuclear antigen positive. All of the tumors derived from carcinogen-treated BS cells still retained high SCE levels.

Nude mouse tumors (tumors 1–5) were clearly retransplantable from nude mouse to nude mouse although tumors 6 and 7 were not tested for retransplantation.

**Histological Finding of Nude Mouse Tumor**

**Light Microscopy.** Tumors developed s.c. in nude mice infiltrated into s.c. fatty tissues and underlying striated muscles. Foci of necrosis or microcystic degeneration were found in tumor tissues. Tumor cells were closely packed and medium to large. The cells had abundant cytoplasm that stained amphophilic with hematoxylin and eosin, deep blue with Giemsa, and pyroninophilic with methyl green-pyronin stain. The nuclei were large and round to ovoid, were occasionally irregular in shape, and contained prominent nucleoli. Mitotic figures were frequently seen (Figs. 3 and 4). Silver impregnation showed a moderate amount of reticulin fibers among the tumor cells. Histologically, there were no differences between the tumors originated from MNNG-treated cells (Tumor 8-I) and those from 4NQO-treated cells (Tumor 3). No tumor metastasis to axillary and inguinal lymph nodes and to the other organs was found.

**Electron Microscopy.** The tumor cells had a round, oval, or...
MALIGNANT LYMPHOMA ANTIGEN EXPRESSED IN BS CELLS

Fig. 5. Electron micrograph showing binucleate tumor cell originated from MNNG-treated human B-cells with broad rim of cytoplasm and prominent nuclei (NU). The cytoplasm contains abundant polyribosomes and a few narrow strands of rough endoplasmic reticulum. Lipid droplets (L). Same tumor as shown in Fig. 3, × 6000.

Fig. 6. Electron micrograph showing tumor cell originating from 4NQO-treated human B-cells. The cell has round nucleus with prominent nucleoli (NU). The cytoplasm contains abundant polyribosomes, swollen mitochondria, poorly developed rough endoplasmic reticulum, and a few lipid droplets (L). Same tumor as shown in Fig. 4, × 8000.

 Occasionally indented nucleus; finely dispersed chromatin; and prominent nucleoli. The cytoplasm contained abundant polyribosomes, several swollen mitochondria, and a few lipid droplets. Endoplasmic reticulum and Golgi apparatus were poorly developed (Figs. 5 and 6). However, some neoplastic cells had well developed Golgi apparatus and rough endoplasmic reticulum with dilated cisternae. Some tumor cells showed plasmacytoid differentiation. Multinucleate tumor cells were also found in small numbers. Epstein-Barr virus or virus-like structures were not demonstrated in tumor cells. Desmosomes or desmosome-like structures were not found between the tumor cells. These findings indicated that most tumor cells in s.c. tissues of nude mice were lymphoid malignant cells.

ML-associated Antigen and Serum Reaction

Since 8-I-derived tumor was constantly and successively transplantable from nude mouse to nude mouse and histopathological findings revealed the characteristics of lymphoid malignancy, cell surface antigens were examined by the IF technique in the reaction with antibody in the sera of ML and related disease patients (Fig. 7). A part of tumor has been successively recultured in vitro, retaining ML-associated antigen. Table 3 lists the whole sera tested. Table 3 and Fig. 8 show the reactions of BS-SYi, TM cells with the sera of healthy, ML, ML-related diseases, leukemia, OVC, and ST patients in IF technique. As shown in Fig. 8, BS-SYi, TM cells showed an approximately similar level of positive reaction to T-cell-type ML, ATL, B-cell-type ML, and Hodgkin's lymphoma cases in over 76% examined, although the positive cell percentages (IF) varied from case to case from 6 to 40% and were more or less low in acute lymphoblastic leukemia and chronic lymphoblastic leukemia. The mean values for IF positive percentages in each case were 26.8 ± 3.21 in T-cell-type ML, 21.2 ± 2.66 in ATL, 25.1 ± 2.34 in B-cell-type ML, 16.2 ± 2.88 in Hodgkin's lymphoma, as compared to 3.88 ± 0.78 (P < 0.001) in ATL carrier, 3.67 ± 0.66 in chronic myeloid leukemia, 3.59 ± 0.91 in acute myeloid leukemia, 3.79 ± 0.61 (P < 0.001) in OVC, and 3.91 ± 0.51 in ST (P < 0.001). In general low levels of IF reaction were found in the serum of healthy blood donors; the level of 5% IF positivity (Fig. 8) (mean of normal subjects + 2 SD) was used to estimate the upper limit of normal sera. Therefore, using an arbitrary cutoff level, sera which gave more than 5% IF positivity were considered to be abnormal; approximately 2 of 165 normal individuals had raised IF positivity in the reaction with BS-SYi, TM cells (Fig. 8). No disease was detected in these two
MALIGNANT LYMPHOMA ANTIGEN EXPRESSED IN BS CELLS

Fig. 8. Levels of ML antigen-positive cells among BS-SY, TM cells in the reaction with sera of patients (normal, ML and related diseases, leukemia, ovarian cancer, and stomach cancer). T-ML, T-cell-type ML; B-ML, B-cell-type ML; HL, Hodgkin’s lymphoma; ALL, acute lymphoblastic leukemia; CLL, chronic lymphoblastic leukemia; CML, chronic myeloid leukemia; AML, acute myeloid leukemia.

Fig. 9. Western blotting analyses based on the reaction of BS-SY, TM cell-derived ML antigen and sera from 14 cases of ML (5 T-cell type (Strips 1-5), 5 B-cell type (Strips 6-10), and 4 ATL (Strips 11-14)), and from 5 normal subjects (Strips 15-19). ML antigen is characterized by a single band at M, 97,000 (97KD). No special bands were detected from Strips 15-19 over M, 60,000 in the reaction of ML antigen and normal sera. Strips 20 and 21 exhibit the negative reaction of ML antigen and two sera in which the ML antibody is taken by the absorption described above.

Fig. 10. Karyotype of ML antigen positive cell (BS-SY, TM) showing 46,XY, t(6;7)(p25;q22), t(9;7)(q34;q34), del(10)(p13), t(12;14)(q24;q11).

Western Blots for ML Antigen

Fig. 9 shows WB analyses in the reaction with 14 ML (5 T-cell, 5 B-cell, and 4 ATL) and 4 normal sera. As seen from Fig. 9, there were characteristically common antigen bands at M, 97,000 for ML, although bands below M, 60,000 reacted with using 5% positive as the dividing line. If this patient develops ATL it would tend to confirm the hypothesis that 5% is the threshold. IF-positive cases were detected in the serum of 1 of 20 OVC patients and 2 of 25 stomach cancer patients, but generally at lower levels than those found in patients with ML and related diseases. No case of 20 patients with fever of unknown origin, possibly due to infection, showed a positive level over 5%. Variable levels of IF positivity might be due to the different quantity of the ML antibody among different cases, although the significance of the low (below 5%) readings of some ML and ATL patients is not clear. It may be due to the difficulty of keeping sera for long periods (more than 2 years). These findings indicate that the antigens of a certain population of BS-SY, TM cells are specific to the serum antibody of ML and related malignancies and valuable for serodiagnosis.
some unknown component of normal sera and were not usable for this diagnostic analysis. In the present WB analysis, 35 of 40 ML cases clearly showed positivity in M, 97,000 band. Membrane proteins collected from antigen-negative cells did not exhibit any specific antigen bands over M, 60,000 area in WB analysis, paralleling the IF procedure. In the lectin reaction staining, ML and ST cancer antigen bands reacted with WGA, although these antigen bands did not react with other lectins (concanavalin A, Dolichos biflorus agglutinin, Lens culivaris agglutinin, phytohemagglutinin-E4, peanut agglutinin, Ricinus communis agglutinin, Ulex europaeus I, WGA). Notable is the fact that M, 97,000 ML band did not react with other cancer sera (OVC 20 cases, LI 10 cases, and LU 10 cases) or with (>60) normal subjects' sera. In the reaction of the absorbed ML serum and strips for WB, no antigen band was detected at M, 97,000 band region. These results strongly indicate that the present WB analysis using 4–20% gradient gel enable distinguishing ML antigen at the M, 97,000 band.

Specificity Analysis of the ML Antigen

To determine whether BS-SY1 TM cell-derived ML antigen was specific for ML, serum (B- or T-cell type) known to contain a high antibody titer (1:100 in immunofluorescence technique) was absorbed with packed BS-SY1 TM cells and restested for residual reactivity with the BS-SY1 TM cells. The BS-SY1 TM cell-derived antigen (1 × 10⁸ cells) was found to absorb completely antibody from both B- and T-cell type lymphoma sera, although the absorption was not enough when less than 5 × 10⁷ cells were applied for the absorption of antibody in sera. When nontreated KS86 and BS-SY1 (Table 1) cells were applied to the reaction as antigens, these cell lines did not reduce the antibody reactivity even if the absorption was repeated. These findings strongly support that the BS-SY1 TM cells carry antigen specific for ML.

Chromosome Analysis of Nude Mice Tumor

Chromosome analysis performed with G- and Q-banding techniques showed a basically normal karyotype (46,XY) in the original BS-SY1 cell line, although chromosome instabilities including breaks (5–8%) and quadriradials (0.01–0.02%) were detected at relatively low frequency in BS-SY1 cells.

In all of tumors, no normal (diploid) human cells were encountered, i.e., all the karyotypes observed had chromosomal changes of the nature shown in Table 2. All tumors resulting from BS-SY1 TM cells showed karyotype abnormalities involving certain marker chromosomes t(1;2)(p11;q11), t(1;4)(p11;q36), t(6;7)(p25;?), t(9;?) (q34;?), del(10)(p13), t(12;14)(q24;q11), even though the untreated cell line had basically a normal karyotype. The development of the abnormal karyotypes was associated with the process of malignant transformation caused by exposure to carcinogens. Several different cell clones with different marker chromosomes were observed at 20–30 days after carcinogen treatment and some of them were noted as the tumors after inoculation onto the s.c. tissue of nude mice. When tumor antigen characteristics were examined with IF using a piece of all direct tumor tissues, ML-associated antigen was detected only in cell membranes of two tumors [tumors 3 and 8-1 (Table 2)] showing t(12;14)(q22;q32) in tumor 3 and t(12;14)(q24;q11) in tumor 8-1; a cell clone which did not contain No. 14 marker did not show ML antigen activity. Karyotype analysis of tumor 8-1 showed that 25 cells were 46,XY,t(6;7)(p25;?),t(9;?) (q34;?),del(10)(p13), t(12;14)(q24;q11) (Fig. 10). Karyotype analyses of tumors 8-II and III, which were successfully transplanted to second and third passages of nude mice, also showed 46,XY,t(6;7)(p25;?), t(9;?) (q34;?),del(10)(p13),t(12;14)(q24;q11). Therefore, the acquisition of t(12;14)(q24;q11) might play a key role for the development of tumor and the expression of ML-associated antigen.

DISCUSSION

Human malignant cells transplanted into nude mice will be potentially very important for human cancer research, particularly for evaluating the in vivo efficacy of chemotherapy. In the present work, we have established the successive transplantation of Bloom’s syndrome cell-derived tumors into nude mice and have detected the strong expression of ML-associated antigen on the cell surface of the tumors. Cell clones with ML-associated antigen would have been selected during the course of the development of the nude mice tumors, although carcino-}

4 Y. Shiraishi, unpublished data.
panied when chromosomal translocation took place between 14q11 and 12q24 cannot be ruled out, the chromosomal re-arrangement in the region involving 14q11 in BS-SY, TM cells might play a pivotal role of an expression of the common antigen associated with T- and B-cell tumors. High frequencies of quadriradials and increased SCEs which were detected in carcinogen-treated BS cells shortly after treatment (1, 2), both equal and unequal could contribute to the karyotypic changes and expressions of oncogene and cancer antigens that we have observed. Establishment of a monoclonal antibody against the ML antigen originated from carcinogen-transformed BS cells is also under study and may facilitate both diagnosis and the analysis of oncogene products.

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