Establishment and Characterization of Transplantable Tumor Derived from a Spontaneous Malignant Fibrous Histiocytoma in the Mouse

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Abstract: A tumor developed spontaneously in the subcutaneous tissue of the hind leg of a 7-month-old female ddY mouse. Light and electron microscopical examinations revealed that the original tumor was composed of an admixture of fibroblast-like and histioyte-like cells arranged predominantly in a storiform or cartwheel pattern. The tumor cells gave positive reactions for acid phosphatase, N-acetyl-β-glucosaminidase, non-specific esterase, β-glucuronidase, alpha-1 antitrypsin and fibronectin. The original tumor was diagnosed as a malignant fibrous histiocytoma (MFH). The tumor was serially transplanted into syngeneic mice up to the 92nd generation. The tumor was also consistently transplanted into allogeneic mice of several inbred strains. The allogeneic mice used in the present study were strains having different H-2 haplotypes. During succeeding passages, transplanted tumors showed aberrant growth properties. The tumor transplanted into mice of inbred strains took well to back transplantation for mice of original strain and allotransplantation for other inbred strains. The pathological features of these transplantable tumors were basically similar to those of the original tumor. As mentioned above, a MFH developed spontaneously in the ddY mouse was consistently transplantable into both syngeneic and allogeneic mice.

Key words: allogeneic transplantation, H-2, inbred mouse, malignant fibrous histiocytoma, spontaneous tumor

Introduction

Malignant fibrous histiocytoma (MFH) is a pleomorphic sarcoma occurring in the soft tissue or bone, and consists of fibroblast- and histioyte-like cells arranged predominantly in a storiform or cartwheel pattern as the most conspicuous feature [3, 29]. As for the origin of MFH, variously divergent views have been presented, and are categorized into the following three: fibroblastic [10, 12], histiocytic [12, 24, 37] and undifferentiated mesenchymal cell [4, 8, 20] origins, but the pathogenesis of MFH was seriously questioned in view of the

(Received 31 May 1995 / Accepted 31 August 1995)
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results of morphologic [4, 12, 26, 28, 32], functional [25] and experimental [10, 37] studies. Because of this controversy, appropriate animal models of induced [11, 14, 37] and spontaneous [34, 36] MFHs are needed for studying the pathogenesis of this malignant tumor.

In general, the recipient animals using in serial transplantation of various tumors are syngeneic animals [11, 33, 36], athymic nude mice [21], immunocompromised mice [19] and immunocompromised newborn hamsters [30] or rats [29]. It is generally thought that the rejection of tumors in allogeneic or xenogeneic animals is dependent on the expression of the major histocompatibility complex (MHC) in the transplanted tumors [2, 17, 22].

Recently, we encountered a spontaneous MFH showing a typical storiform pattern on histopathological examination. It occurred in the left hind leg of a 7-month-old female mouse of ddY strain. Interestingly, this MFH tumor was successfully serially transplanted into both syngeneic mice of ddY strain and allogeneic mice of several inbred strains. There have been few reports on the allogeneic transplantation of malignant tumors [16, 33].

In this paper, morphological properties, allotransplantability and characteristics of tumor growth derived from a MFH developed spontaneously in the ddY mouse are described and discussed from the point of view of the tumor-host relationship.

Materials and Methods

Animals: For syngeneic transplantation of tumor, ddY mice of both sexes, which had been purchased from JAPAN SLC Co. or produced in the our laboratory, were used throughout the studies. For the experiment of allogeneic transplantation, inbred mice of 7 strains, A/J, AKR/N, BALB/c, C3H/He, C57BL/6, DBA/2 and MRL/lpr (all from JAPAN SLC Co.), were used. H-2 haplotypes of these mice are shown in Table 1. On the other hand, Hartley guinea pig, Wistar and SD rats and Syrian hamster (all from JAPAN SLC Co.) were used for xenogeneic transplantation control. All animals used in this study were 5-week-old males and females. The animals were maintained in barrier rooms conditioned to 23 ± 1°C and 55 ± 10% relative humidity, and given an F2 diet (Funahashi farm Co.) or CG-3 diet (CLEA JAPAN Co.) with water ad libitum.

Transplantation: Transplantation to syngeneic ddY mice, allotransplantation in inbred mice and back transplantation to ddY mice from inbred mice were examined. Transplantable mammary tumor (BTM-1) in BALB/c mouse was used as the negative control. Ten animals were used as recipients for transplantation of tumors. Treatment of tumors for transplantation were performed as follows. The tumors were surgically excised from donor mice under aseptic conditions. The tumor tissue was minced and placed in refrigerated Hanks’ balanced solution containing penicillin (1 × 10⁵ unit/1,000 ml) and streptomycin (100 mg/1,000 ml). One fragment about 1 mm in diameter was implanted with a trocar in the subcutaneous tissue of the back of each recipient animal. After the transplantation, transplantation sites were observed every day, and the tumors were measured weekly. When the tumor in an animal selected at random from among 10 animals reached about 20 mm in diameter, it was used for serial transplantation. The remaining 9 animals were not used for the serial transplantation, and the tumor was measured until they died. These animals were used for analysis of tumor growth.

The estimates of tumor growth were performed as follows. To determine tumor doubling time, estimates of tumor volume (V) in mm³ were calculated with the formula: \(V = a \times b^2/2\), where a is the width in mm and b is the length in mm. The data obtained for exponentially growing tumors were analyzed by linear regression techniques, and the doubling time was calculated from the \(\ln 2/slope\).

Pathological examination: For histological observation, the residual tumor tissues and major organs were fixed in 10% buffered formalin, and sections were routinely stained with hematoxylin and eosin (H-E). Special stains, such as periodic acid-Schiff, azan Mallory, alcian blue and silver impregnation, were used when necessary.

For histochemical observation, fresh tumor tissues obtained at surgery were fixed in 4% formol calcium (pH 7.1) for 1–8 hr at 4°C. The tumor tissues were then washed two or three times with cold distilled water and stored for 24 hr at 4°C in Holt’s hypertonic gum sucrose solution (0.8M sucrose containing 1% gum acacia). The tumor tissues were then frozen, and about 7 μm-thick sections were cut. The enzymes examined were alkaline phosphatase (AH-P; Gomori method), acid
phosphatase (Ac-P; Burstone’s method), N-acetyl-β-glucosaminidase (N-GA; Glycosidase method), β-glucuronidase (β-GL; Fishman method) and non-specific esterase (N-SE; Thiocoline method).

For immunohistchemistry, alpha-1-antitrypsin (α1-AT; ART Co.) and fibronectin (FN; UCB Co.) were used to study the origin of the tumor cells. MHC (H-2d and H-2k; Seikagaku Corporation Co.) was used to distinguish the MHC class of the tumor cells. These immunostainings were performed by the peroxidase-antiperoxidase (PAP) method. Normal mouse sera were used as negative controls.

For electron microscopy, specimens were processed routinely and examined with a JEOL’s model JEM-100SX electron microscope.

**Results**

Pathological findings of original tumor: Grossly, the original tumor occurred in the subcutaneous tissue of the left hind leg of a 7-month-old female ddY mouse, which was under observation of its life span. When discovered, the tumor was a solid nodule, 14 × 13 × 10 mm in size and weighed 8 g. The tumor was hard and encapsulated. But this capsular border was partially broken, and the tumor mass invaded the surrounding tissue. The cut surface of the tumor was yellowish-white in color, and was multi-lobular with a fascicular structure.

Histologically, the tumor was composed of a mixture of plump fibroblast-like cells and rounded histiocyte-like cells arranged in a storiform or cartwheel pattern (Fig. 1). A moderate amount of collagen fibers was present throughout the tumor tissue. The tumor cells contained PAS-positive materials in their cytoplasm. Reticulin fibers in the central area of the storiform arrangement were seen clearly following silver impregnation. Bizarre giant cells were rarely found. Mitotic figures were frequent. No metastatic lesions were found in major organs.

In the histochemical and immunohistochemical examinations, tumor cells gave faintly to moderately positive reactions for N-GA, β-GL, N-SE and FN, and strongly positive reaction for Ac-P (Fig. 2) and α1-AT (Fig. 3), but Al-P was not demonstrated in the tumor cells. The reactivities of Ac-P and α1-AT in particular were demonstrated in the cytoplasm of histiocyte-like and giant cells, and β-GL activity was also demon-

<table>
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<tr>
<th>Recipient Strain</th>
<th>H-2 Haplotype</th>
<th>Passage</th>
<th>Incidence of tumor* (%)</th>
<th>Tumor-take time** (Days)</th>
<th>Doubling time (Days)</th>
<th>Relative tumor weight (%)</th>
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<td>A/J</td>
<td>a</td>
<td>1-16</td>
<td>210/210 (100)</td>
<td>3.8±0.7</td>
<td>1.8±0.5</td>
<td>46.2±5.9</td>
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<td>AKR/N</td>
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<td>1-21</td>
<td>199/210 (93)</td>
<td>4.2±0.6</td>
<td>1.9±0.6</td>
<td>41.0±8.8</td>
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<td>1-46</td>
<td>457/460 (99)</td>
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<td>1.8±0.9</td>
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</tr>
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<td>1-29</td>
<td>290/290 (100)</td>
<td>3.3±0.9</td>
<td>1.6±0.9</td>
<td>43.9±4.8</td>
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<td>C57BL/6</td>
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<td>1-23</td>
<td>230/230 (100)</td>
<td>2.8±0.7</td>
<td>1.7±0.5</td>
<td>42.3±6.5</td>
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<td>DBA/2</td>
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<td>250/250 (100)</td>
<td>3.8±0.9</td>
<td>1.9±0.7</td>
<td>41.3±4.3</td>
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<td>1.3±0.2</td>
<td>54.4±3.2</td>
</tr>
<tr>
<td>MRL/lpr</td>
<td>k</td>
<td>1-21</td>
<td>130/130 (100)</td>
<td>2.8±0.9</td>
<td>1.5±0.8</td>
<td>44.9±5.2</td>
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</table>

Serial transplantation MFH tumor pieces were inserted into subcutaneous tissue of the back. * Number of tumorigenic mice/number of treated mice. ** Tumor-take after transplantation judged by palpation.
Electron microscopy revealed four differentiated cell types: fibroblast-like, histiocyte-like, giant and undifferentiated cells (Fig. 4). Fibroblast-like cells were characterized by spindle-shaped and elongated cytoplasm, and were associated closely with collagen fibers. The cytoplasm contained dilated rough-surfaced endoplasmic reticulum, numerous mitochondria and well-developed Golgi apparatus. Histiocyte-like cells had abundant cytoplasm with a profusion of surface folds and horseshoe-shaped nuclei. Cytoplasmic organelles comprised short profiles of rough endoplasmic reticulum, numerous lysosomes and well developed Golgi apparatus. Giant cells showed variant forms of well differentiated histiocytic cells; they commonly contained many vacuoles of various size, abundant microvilli and mono or multi nuclei of markedly irregular shape. Undifferentiated cells were small and oval with an irregular cell surface. The cytoplasm was poorly developed except for numerous free ribosomes and some mitochondria. Their nuclei were round or oval, and the nucleocytoplasmic ratio was high.

Transplantation of tumor: The transplantability of the tumor was not significantly different in males and females. The results for both sexes are therefore presented together. The growth curve for a transplantable tumor in syngeneic and allogeneic mice is shown in Fig. 5. Serial transplants from the original tumor grew well in syngeneic mice of the ddY strain. And the tumor grew in all allogeneic mice of the 7 inbred strains after the
Fig. 3. Immunohistochemical view of a transplantable tumor in the C3H/He mouse. Positive reaction with α1-AT is seen in rounded histiocytic cells (white arrows) and giant cells (black arrow). PAP method, counterstained with methyl green. × 200.

Fig. 4. Electron microscopic view of the original tumor in the ddY mouse. Typical fibroblast-like (f), histioyte-like (h), giant (g) and undifferentiated (u) cells with collagen fiber (co) are observed. × 1,800.

Table 2. Histochemical and immunohistochemical characteristics of transplantable tumor derived from a spontaneous subcutaneous tumor of ddY mouse

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Ac-P</th>
<th>Al-P</th>
<th>N-SE</th>
<th>β-GL</th>
<th>N-GA</th>
<th>GAG</th>
<th>Collagen</th>
<th>Silver</th>
<th>FN</th>
<th>α1-AT</th>
<th>H-2d</th>
<th>H-2k</th>
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<td>Transplantable tumor*</td>
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+: All cells negative, ±: Less than half of the cells positive, +: Half of the cells positive, ++: More than half of the cells positive.
transplantation of tumor tissue from ddY mouse. As shown in Fig. 5, however, in contrast to syngeneic mice, in allogeneic mice there was a retardation of tumor growth from 30 days after transplantation. The transplantability of the tumor to syngeneic and allogeneic mice is shown in Table 1. The tumor was serially transplanted into syngeneic mice of the ddY strain up to the 92nd generation and into allogeneic mice of 7 inbred strains until the 16th to 46th generations. The transplantability of the tumor in recipient mice of each strain ranged from 93% to 100%. The mean tumor take time in syngeneic mice was 2.3 days, and in allogeneic mice it was 3.4 days. The shortest doubling time was 1.3 days in the syngeneic mice, and 1.5 days in the allogeneic mice. The relative tumor weight ranged from 41.0% to 54.4%. No significant difference between syngeneic and allogeneic mice was noted in the transplantability of the tumor. BTM-1 transplantable mammary tumors were not transferable to allogeneic mice, and xenogeneically transplanted tumor of the ddY mouse origin took temporarily in SD rats and Syrian hamsters.

As Table 3 shows, back transplantation of the present tumor from mice of 7 inbred strains took well in mice of the original ddY strain, and the growth of tumors obtained in mice of inbred strain were generally better than that in mice of other different inbred strains. On the other hand, back transplantation from xenogeneic Sprague-Dawley rats and Syrian hamsters was successful in ddY mice. 

Pathological findings of transplantable tumors: Grossly, well-defined solid nodules adhered firmly to the epidermis of the skin in recipient mice (Figs. 6a and 6b). Some recipient mice bearing large tumors died 8 to 17 weeks after transplantation, showing signs of depression and emaciation. The cut surface of the tumors almost invariably showed central necrosis and infiltrative borders. Immunohistochemically, positive reactions for H-2d and H-2k antigens were not demonstrable in proliferating tumor cells in recipient mice (Table 2).

On the other hand, histological, histochemical, immunohistochemical and electron microscopical findings for all transplantable tumors retained the characteristics of the original tumor in the ddY mouse.

**Discussion**

Although various spontaneous neoplastic lesions in mice have often been reported, there are few papers describing MFH in mice [27]. However, experimental MFH in mice have been induced by inoculation into syngeneic mice of bone marrow or peritoneal macrophages transformed by SV-40 [6, 37]. We found the tumor developed spontaneously in a ddY mouse. The tumor was composed mainly of fibroblast-like, histiocyte-like, giant or undifferentiated cells arranged in a storiform pattern. The tumor cell populations in the human MFH have been shown to consist of the following five major cell types: fibroblast-like, histiocyte-like, xanthomatous, giant and undifferentiated cells [4, 8]. In the present tumor, we observed mainly fibroblast-like and histiocyte-like cells, and giant and undifferentiated cells were often seen in focal areas in a storiform arrangement. But no xanthomatous cells were seen. These findings suggest the typical architec-
Table 3. Summary of back transplantation for allogeneic and syngeneic mice of tumor derived from a spontaneous malignant fibrous histiocytoma in ddY mouse

<table>
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<tr>
<th>Recipient</th>
<th>Donor / Tumor</th>
<th>ddY</th>
<th>A/J</th>
<th>AKR/N</th>
<th>BALB/c</th>
<th>C3H/He</th>
<th>C57BL/6</th>
<th>DBA/2</th>
<th>MRL/lpr</th>
<th>Hartley</th>
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MFH: Transplantable tumor derived from spontaneous malignant fibrous histiocytoma in ddY mouse. BTM-1: Transplantable tumor derived from mammary cancer in BALB/c mouse origin.


ture and cytologic features of MFH. In the histochemical and immunohistochemical findings, tumor cells gave faintly to strongly positive reactions for Ac-P, N-GA, N-SE, β-GL and α₂-AT, suggesting that the tumor cells may be of histiocytic origin. Positive reactivities for these enzymes and α₂-AT markers have frequently been demonstrated in human MFH, although the degree of reaction varied from case to case. FN is known to be a secretory product of many types of cells, including endothelial and fibroblastic cells. Watanabe et al. [31] reported that alveolar macrophages were positively stained by FN. In this study, among the fibroblast-like, histiocyte-like and giant cells were moderately stained. On the basis of pathological examination of a spontaneous tumor in the ddY mouse, it appears that this tumor is similar morphologically to human MFH [28], and was diagnosed as MFH.

The syngeneic or athymic nude mice have generally been used as recipients in an experimental model of transplantation of tissues [30]. However, few such models of the transplantation of host tumors to allogeneic animals have been reported [16, 33]. In the MFH transplantation models, many attempts have been made to transplant MFH into syngeneic animals [35, 36] or nude mice [9]. We succeeded in allotransplantation from a tumor of ddY mouse origin to mice of several inbred strains. The tumor grew well in allogeneic mice of 7 inbred strains. The transplantable tumors maintained the pathological characterization of the original tumor in the ddY mouse. Similar results have been reported by Kosmehl et al. [15] by inoculating allogeneic mice with a piece of 20-methylcholanthrene-induced tumors in NMRI mice.

Many inbred strains of mouse have been established and used in various immunological studies [7]. It has been reported that mice carrying H-2 haplotypes are susceptible to transplantation of tissues [1, 18]. H-2 haplotypes of inbred mice used in this study were H-2a (A/J), H-2b (C57BL/6), H-2d (BALB/c and DBA/2) and H-2k (AKR/N, C3H/He and MRL/lpr) [1, 13, 23]. But, the H-2 haplotype of the ddY mouse is not known. It was also noted that the DDD/1 mouse is supposed to
Fig. 6. Macroscopical view of tumors transplanted into mice of several strains. a. Left to right; ddY, C57BL/6, AKR/N and C3H/He mice. b. Left to right; BALB/c, A/J, MRL/lpr and DBA/2 mice.

have H-2s [5]. It is therefore possible that the H-2 haplotype in the ddY mouse is H-2s. In this study, a spontaneous tumor in the ddY mouse was consistently transplantable into both syngeneic and allogeneic mice. These results revealed that mouse strain-related differences in take-response to transplantation of the present tumor did not relate to their H-2 haplotypes. In the immunohistochemical examination, the positive reactivities of anti H-2d and H-2k were observed on lymphocytes in BALB/c, DBA/2, C3H/He, AKR/N and MRL/lpr mice used as the positive control, whereas the transplantable tumor cells in syngeneic and allogeneic mice were completely negative. This negative reaction for transplantable tumors in allogeneic mice strongly
suggest that the present tumor cells are not related to the H-2d or H-2k antigen. Allogeneic transplantation of BTM-1 of BALB/c mouse origin used as the control tumor resulted in rejection in all syngeneic mice. In an immunohistochemical examination, the BTM-1 tumor cells were shown to be positive for the H-2d antigen. It is conceivable that the possible mechanisms may be caused by the production of blocking factors from tumor cells of ddY mouse origin rather than by the histocompatibility response of different H-2 haplotypes to tumor pieces transplanted in allogeneic mice [18]. But, the present study failed to clarify details of the mechanism of allotransplantation of the tumor used.

In further studies, detailed investigation of biological characteristics, immunological effects and the existence of blocking factors in the tumor used in this study will be necessary to analyze this phenomenon. We believe that this allotransplantable tumor may provide a useful tool for basic studies of tumor associated transplantation antigen. Work is now in progress to further elucidate these mechanisms.

References

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