A New Transplantable Mouse Liver Tumor of Spontaneous Origin

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Summary

A transplantable primary liver tumor 1st observed in a 2-month-old female Swiss-Webster mouse is described. Morphologically, it was poorly differentiated. It was easily converted into an ascitic form. The tumor grew rapidly and uniformly without regression in 100% of Swiss-Webster mice after the 2nd transplant generation. Several characteristics of this tumor distinguish it from other transplantable primary liver tumors of the mouse; growth was rapid and invasive, and by Generation 4 no host-conditioning (X-irradiation and/or cortisone) was required; strain specificity was minimal, with progressive tumor growth in 100% of C3HeB/FeJ, DBA/2J, C57BL/6 Millerton, AKR/J, and C56BR/cdJ inbred, and in ICR/Ha Swiss random-bred mice.

Introduction

Variability in the incidence of spontaneous primary liver tumors in mice has been reported. The highest incidences in inbred mice were observed in the C3H and CBA lines. Andervont (1) found an incidence of 15–55% in C3H and 20–29% in CBA mice. In recent studies, Heston et al. (7), reported an incidence of 85% in C3H male mice and Williams and Bonser (13) reported an incidence of 11% in CBA male mice. Spontaneous liver tumors typically arose only in mice beyond 12 months of age (1, 3, 7, 10, 13) and were more frequent in male than in female mice (1, 10, 13). A spontaneous liver tumor incidence of 5% was seen in the wild house mouse, Mus musculus (2). An incidence of 88–100% was observed in F1 hybrid mice, offspring of C3H x YBR, maintained to the advanced age of 16 months (6). No liver tumors were found in 784 random-bred Swiss-Webster mice observed to the age of 1 year (8).

This report describes a spontaneous, transplantable primary liver tumor originating in a 2-month-old female Swiss-Webster mouse. Several features distinguish this tumor from other mouse liver tumors and suggest that it may be suitable for various experimental studies.

Materials and Methods

Young adult, 2-month-old, random-bred Swiss-Webster mice weighing 19–22 gm were obtained from Taconic Farms, German-town, New York. Random-bred Swiss ICR/Ha and inbred C57BL/6 mice were purchased from Millerton Research Farms, New York; all other inbred mice were obtained from the Jackson Laboratory, Bar Harbor, Maine. Only female mice were used.

In early transplant generations a modification of the host-conditioning procedure of Teller et al. (12) was used. Cortisone acetate was injected s.c. on Day 0 and Day 2 of transplantation, and X-radiation was given as a single total body dose prior to implantation of tumor tissue. All mice in the 1st 4 transplant generations were examined at least once weekly. Tumor growth in these groups was confirmed by autopsy; all growths (takes) were progressive in mice held for long-term observation. Sections were prepared and stained with hematoxylin and eosin for microscopic examination.

The tumor was transplanted i.m., s.c., i.p., and intrahepatically. A suspension of minced tumor tissue was used for both the i.m. (the deep femoral muscle of the left hind leg) and the liver transplantations. The solid tumor was minced with scalpels and prepared as a 50% suspension in Locke-Ringer’s solution, containing 0.6% glucose and 1% McIlvaine’s citric acid-sodium phosphate buffer at pH 7.4 (BGR). A dose of 0.2 ml of this suspension was injected with a hypodermic needle and syringe. Liver transplantations were made into anesthetized mice after surgical exposure of the livers. Two-mm cubes of tumor tissue were implanted s.c. into the right flank by trocar. Ascitic tumor cells were removed from the peritoneal cavity, counted, appropriately diluted with BGR, and injected i.p. in a 0.2-ml volume. Solid tumors were maintained in the intramuscular site.

Tumor Origin and Morphology

The liver tumor was 1st observed (by H. S. Taper) in a 2-month-old female Swiss-Webster mouse from Taconic Farms. No tumors had been noted before this in over 2000 similar mice from the same source. The primary tumor was 1st detected as a palpable abdominal mass, which subsequently grew rapidly in size. Fourteen days after the initial observation, the moribund mouse was killed and examined.

At autopsy, a single globular 3-gm tumor was disclosed in 1
1

2

3

4

5

6
of the lobes of the liver. Grossly, the surrounding tissues appeared normal. The tumor was yellowish-white, soft, friable, and limited by a fibrous capsule 1–2 mm thick. No metastases or abnormalities of other organs were observed.

The most viable-appearing areas of the tumor were used for transplantation to other mice, and only small samples of the tumor and capsule were left for histologic and pathologic studies. Microscopic examination revealed almost completely necrotic masses together with small groups of poorly differentiated multiangular and round cells. The capsule surrounding the tumor was composed of fibrous connective tissue with scattered inflammatory cells and groups of proliferating multangular epithelial cells, which formed trabeculae closely attached to the sinuses (Fig. 1). The cytoplasm of the multangular epithelial cells was uniform, abundant, and slightly basophilic. The nuclei of these cells were variously enlarged and hyperchromatic and sometimes exhibited prominent nucleoli. These formations closely resembled liver structure. Other areas composed of more uniform and less differentiated cells scattered in the fibrous connective tissue of the capsule were also found (Fig. 2), and some groups of these cells were seen invading the walls of large blood vessels (Fig. 3). In no instance were formations observed that were reminiscent of a liver diet system. Fig. 4 is representative of the structure of the first transplant generation (T-1). The tumor cells were more pleomorphic, with numerous mitotic figures and abundant cytoplasm. The cordlike arrangement was less prominent. The cells were much larger than average and occasionally contained 2–3 vesicular nuclei. The pleomorphic neoplastic cells formed occasionally trabeculae structures situated close to sinusoidal spaces (Fig. 5). In the 3rd transplant generation (T-3), marked uniformity of histologic pattern was observed (Fig. 6). Tumor spaces (Fig. 5). In the 3rd transplant generation (T-3), marked uniformity of histologic pattern was observed (Fig. 6). Tumor cells were rounded and individually placed and have numerous mitotic figures. X 440.

CHART 1. Transfer record of the liver cell tumor in conditioned (X-irradiation and/or cortisone acetate) and nonconditioned Taconic Swiss-Webster female mice. X1, 150 r X-irradiation, single total body dose; X2, 100 r X-irradiation, single total body dose; C, cortisone acetate, 6 mg denoting total dose in 2 s.o. injections, and 3 mg denoting total dose as 1 s.o. injection; d, age of growth in days when tumor was removed for examination or transplantation, and the ratio refers to number of tumor takes per number of mice implanted.

Transplantation Studies

CONDITIONING. To assure successful growth of the primary tumor, all mice received 150 r of X-irradiation on the day of implantation and were arranged randomly in 4 groups: (a) implantation s.c.; (b) implantation i.m.; (c) implantation s.c., plus cortisone at 50 mg/kg X 2 on Days 0 and 2; (d) the same as c, but implantation i.m. The last group gave the most successful results.

Fig. 1. Histologic picture of the capsule of the original mouse liver tumor. Multangular epithelial cells form trabeculae closely attached to sinusoidal spaces. The cytoplasm is abundant, uniform and slightly basophilic. The nuclei are variously enlarged and hyperchromatic. X 440.

Fig. 2. Groups of poorly differentiated, epithelial-like cells scattered in the connective tissue of the capsule of the original mouse liver tumor. X 600.

Fig. 3. Poorly differentiated tumor cells invading the wall of a large blood vessel in the peripheral part of the original mouse liver tumor. X 440.

Fig. 4. First generation of mouse liver tumor transplant. Tumor cells are pleomorphic, with abundant cytoplasm and vesicular, hyperchromatic nuclei. Occasional multinuclear cells are present. X 440.

Fig. 5. First generation of mouse liver tumor transplant with pleomorphic neoplastic cells, which formed occasionally trabeculae structures situated close to sinusoidal spaces. X 440.

Fig. 6. Third generation of mouse liver tumor transplant with marked uniformity of the size and shape of the neoplastic cells. Tumor cells are rounded and individually placed and have numerous mitotic figures. X 440.

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BGR was implanted into the thigh of the left hind leg. Six mice
0.2 ml of a 50% suspension of 7-day-old solid tumor mince in
measurements of intramuscular growth were obtained as follows:
by multiplying length, width, and depth for each tumor. Meas
were successful. Growth of 2-cu mm pieces of 7-day-old tumor
inoculum for subsequent transplantation. Smears of the ascitic
was found in 3 of the 5 mice, and these were used as sources of
ponderance of single tumor cells. No clumping of tumor cells
in 4/5 mice (Group d), compared with 1/5, 0/5, and 3/5 in
Groups a, b, and c, respectively (Chart 1). Takes were 2/5 in
T-2 and 5/5 in T-3, including the group conditioned with 100 r
of X-irradiation plus a single 3-mg dose of cortisone (T-3).
By T-4, no conditioning was necessary for 100% tumor takes.
The solid form of the tumor is in its 61st transplant generation
at this time.

CONVERSION TO ASCIITES FORM. For quantitative studies, the
solid tumor was converted into an ascitic form. In an earlier
study, Sato et al. (11) immediately converted only 2 of 16 in-
duced mouse hepatomas. Tumor tissue from T-4 was implanted
i.p. as a tumor mince (0.2 ml of a 50% minced tumor suspension),
and i.p. (5 X 10^4 cells).

In the 1st transplant generation (T-1), tumor growth occurred
in 4/5 mice (Group d), compared with 1/5, 0/5, and 3/5 in
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A Transplantable Mouse Liver Tumor

Effect of Dose on Mortality of Swiss-Webster Mice Implanted with Ascites Cells

<table>
<thead>
<tr>
<th>No. of cells inoculated</th>
<th>Mortality by Day 30 (%)</th>
<th>Mortality of last survivor (day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 × 10^6</td>
<td>100</td>
<td>16</td>
</tr>
<tr>
<td>5 × 10^7</td>
<td>100</td>
<td>19</td>
</tr>
<tr>
<td>2.5 × 10^6</td>
<td>100</td>
<td>20</td>
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<tr>
<td>1 × 10^6</td>
<td>100</td>
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<tr>
<td>5 × 10^5</td>
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<td>&gt;30</td>
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<td>50</td>
<td>&gt;30</td>
</tr>
<tr>
<td>1 × 10^4</td>
<td>40</td>
<td>&gt;30</td>
</tr>
<tr>
<td>5 × 10^3</td>
<td>20</td>
<td>&gt;30</td>
</tr>
</tbody>
</table>

Comparison of Intramuscular Tumor Growth in Inbred and Random-Bred Lines of Mice

<table>
<thead>
<tr>
<th>Mouse line</th>
<th>Av. tumor wt. (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swiss-Webster (Taconic)</td>
<td>2.4 ± 0.6^d</td>
</tr>
<tr>
<td>ICR/Ha Swiss</td>
<td>1.7 ± 0.4</td>
</tr>
<tr>
<td>C3HeB/FvJ</td>
<td>2.0 ± 0.6</td>
</tr>
<tr>
<td>DBA/2J</td>
<td>1.6 ± 0.4</td>
</tr>
<tr>
<td>AKR/J</td>
<td>1.6 ± 0.3</td>
</tr>
<tr>
<td>C57BR/J</td>
<td>1.4 ± 0.4</td>
</tr>
<tr>
<td>C57BL/6 Millerton</td>
<td>0.7 ± 0.2</td>
</tr>
</tbody>
</table>

* 20 mice per line.
^ Mean ± S.D.


discussed, progressively growing tumors in the liver, measuring approximately 1.0 cu cm. No nodules or evidence of any abnormalities was observed in sham-operated mice injected intrahepatically with BGR. Microscopic examination of the tumors growing in the liver revealed cells with the same morphologic characteristics as those seen in Fig. 5, representative of i.m. and s.c. transplants.

5. Several lines of inbred mice were implanted i.m. in the left hind leg with suspensions of minced solid tumor tissue for growth comparisons with 2 random-bred lines, the Taconic Swiss-Webster and the Swiss ICR/Ha. Twenty mice of each line were used, and tumor growth was measured on Day 7. Table 2 shows that growth occurred in all mice, the average tumor weights being significantly smaller than those in Taconic Swiss-Webster mice (P ≤ 0.05).

The C57BL/6 mice were the most resistant on the basis of mean tumor weight attained at 7 days.

Stability in Storage. Tumor viability was determined after storage at −76°C in an alcohol-solid CO2 bath (5) and at −196°C in liquid nitrogen (4). After 4 hr or after storage for 2 weeks, both the solid and the ascitic form of the liver cell tumor, transplanted s.c. and i.p., respectively, demonstrated 100% viability and negligible retardation of growth. After storage for 11 months at −76°C, viability was still 100% for both ascitic cells and solid tumor. However, only 50% viability was demonstrated by each form of tumor stored in liquid nitrogen for 12 months.

Discussion

The spontaneous mouse tumor described has been classified as a primary liver tumor because of localization in the liver and absence of abnormalities in any other organ. The most proper nomenclature for this tumor appears to be “hepatoma,” which would indicate mainly the organ of origin. Novikoff (9) employed the same reasoning for selecting the designation “hepatoma.” At present there are no histologic or histochemical means of diagnosing poorly differentiated tumor cells to confirm hepatocellular origin. Nevertheless, in the early generations of the tumor described, cordlike formations of cells maintaining epithelial features and resembling liver cells were observed. These findings, in addition to the absence of proliferating duct structures, and the absence of any close relationship of tumor cells with mesenchymal elements, as observed on slides stained with Masson’s trichrome and Gomori’s silver staining methods, could indicate the liver cell origin.

It remains undetermined whether the tumor originated from the single aggregates of undifferentiated cells or from the more highly differentiated liver cell formations, both found in the fibrotic capsule.

The histologic pattern of the early tumor compared with later transplant generations indicated a further loss of differentiation. Variations described in transplantable spontaneous hepatomas by other authors have not been found in this tumor. The uniform histologic pattern indicated the proliferation and propagation of cells of a single origin. In histologic structure, high % of tumor takes, and rapid growth, this spontaneous tumor resembles the Novikoff chemically induced rat hepatoma.

The tumor described occurred in a young female mouse of a particular line whose incidence of spontaneous hepatoma is extremely low. Rapid growth in both solid and ascitic forms, uniformity of growth rate and morphologic structure, growth in several recognized strains of mice, and absence of tumor regression distinguish this spontaneous mouse liver tumor from other previously described spontaneous mouse liver tumors. It may thus be useful for oncolgic studies.

Acknowledgments

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