A Model of Malignant Urinary Bladder Tumor in Rabbits

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NEMOTO, R., MORI, H., IWATA, K., KATO, T. and HARADA, M. A Model of Malignant Urinary Bladder Tumor in Rabbits. Tohoku J. exp. Med., 1981, 134 (3), 257-263 —— We attempted to produce a malignant urinary bladder tumor in rabbits by transplanting tumor cells. Each of 51 male mixed-bred rabbits received 0.3 ml V₂ carcinoma cell suspension. The transplantation was done after laparotomy via injection into the bladder wall. Within 2 weeks malignant tumors were observed in the bladder. Without any manipulation to the mucosa, all of the tumors were led to exulceration into the bladder lumen. The incidence of malignant tumor reached 96 per cent. Metastases in these animals were seen in the lungs and para-aortic lymph nodes. This malignant tumor model seems especially suitable for the study of new methods of transurethral treatment of bladder cancer.

One of the problems in experimental studies on bladder cancer consists in a lack of a good animal model large enough to simulate the human situation, especially to study the effectiveness of various transurethral treatments of urinary bladder cancer. Generally, in larger animals, particularly in dogs and rabbits, the tumor induction by usual bladder carcinogens such as aromatic amines has been quite difficult and takes several years before they show an effect (Magee and Barnes 1967; Clayson and Cooper 1970; Cohen et al. 1975). Recently, Harzmann and associates (1979) reported a model of malignant urinary bladder tumor in rabbit by transplanting a suspension of Brown-Pearse carcinoma, which had been developed in a syphilitic scrotum ulcer. In their model, however, cumbersome procedure is necessary to make transplanted tumors proliferate into the bladder lumen. In the present investigation we designed a simple and reproducible model of malignant urinary bladder tumor in rabbits using transplantable V₂ carcinoma.

MATERIALS AND METHODS

A single-cell suspension of V₂ carcinoma was prepared as follows: The V₂ carcinoma is a highly malignant transplantable tumor which originated in a Shope virus-induced papilloma of a domestic rabbit over 40 years ago (Kidd and Rous 1940). A fresh tumor was aseptically excised from the hind limb of a rabbit and trimmed off all fibrous tissues.

Received for publication, August 19, 1980.
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After mincing with scissors, Hanks' balanced salt solution was added to the tumor pulp and the pulp was put through a gauze filter into a sterile dish. The tumor cell suspension was not trypsinized. Viable tumor cell counts of the cell suspension were checked by microscopic study after staining with methylene blue.

Male rabbits (Japanese white) of mixed breed weighing from 2.5 to 3 kg were anesthetized with pentobarbital 25 mg/kg. After the exposure of the urinary bladder through the lower abdominal incision, 0.3 ml of tumor cell suspension was injected into the bladder wall in various situations.

Various numbers of V2 carcinoma cells were injected into the bladder wall of 24 rabbits. The animals were sacrificed 2 weeks after the inoculation and checked for tumor size. Average size of the tumor was calculated using the formula, length \times width mm² (mean±s.E.).

Another group of animals were sacrificed at the determined intervals after the inoculation of \(2 \times 10^6\) cells. All bladders were removed and distended with 10% formalin, and checked for tumor size. After fixation, the bladder tumors were weighed and sectioned for histological study.

**Results**

**Tumor induction**

Table 1 shows the tumor size of the urinary bladder in the rabbits 2 weeks after the transplantation of various numbers of V2 carcinoma cells. Of the 24 rabbits inoculated with more than \(10^5\) cells only 2 rabbits failed to induce the tumor. The remaining 22 rabbits had macroscopically observable bladder tumors. The rabbits which received \(10^6\) tumor cells showed the highest reproducibility in tumor take. While the tumors in the rabbits given \(10^3\) cells and \(10^4\) cells were localized in the submucosal layer, all the tumors in the rabbits given \(10^5\) and \(10^6\) cells were exulcerated into the bladder lumen, and the tumors from \(10^6\) cells underwent central necrosis.

Table 2 shows the tumor frequency and reproducibility after inoculation of various numbers of tumor cells. Of the 51 rabbits that received a tumor cell transplant, only 2 cases resulted in no tumor growth. The tumor takes was thus 96 per cent. Of the 7 rabbits inoculated with \(10^7\) cells, metastasis was found in 6 rabbits at the para-aortic lymph nodes and the lung. All these lungs showed multiple metastases and the size of metastatic nodules was very similar in all animals.

<table>
<thead>
<tr>
<th>Inoculated cell number</th>
<th>Tumor frequency</th>
<th>Tumor size (mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(10^3)</td>
<td>4/6</td>
<td>15.0±3.8</td>
</tr>
<tr>
<td>(10^4)</td>
<td>6/6</td>
<td>88.9±13.7</td>
</tr>
<tr>
<td>(10^5)</td>
<td>6/6</td>
<td>150.8±25.9</td>
</tr>
<tr>
<td>(10^6)</td>
<td>6/6</td>
<td>421.7±25.6</td>
</tr>
</tbody>
</table>

* Number of cells suspended in 0.3 ml of Hanks' buffered saline that were inoculated.

† Calculated using the formula, length \times width (mean ±S.E.).
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Tumor progression and metastases

Two animals each were sacrificed to check for tumor size and histology at weekly intervals after the transplantation of $2 \times 10^6$ tumor cells (Table 3). Histological features of the tumor progression and metastases were as follows:

**Table 3. Progression and metastases of tumors of which histological features were studied**

<table>
<thead>
<tr>
<th>Weeks after inoculation*</th>
<th>Tumor size† (mm × mm)</th>
<th>Extension‡</th>
<th>Metastases</th>
<th>Body weight§ (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lymph node§</td>
<td>Lung/‡</td>
</tr>
<tr>
<td>1</td>
<td>$7 \times 6$</td>
<td>sm to pm1/2</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>$20 \times 19$</td>
<td>m to pm2/3</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>$26 \times 25$</td>
<td>m to $a_0$</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>$33 \times 27$</td>
<td>m to $a_1$</td>
<td>#</td>
<td>#</td>
</tr>
<tr>
<td>5</td>
<td>$45 \times 30$</td>
<td>m to $a_1$</td>
<td>###</td>
<td>###</td>
</tr>
</tbody>
</table>

* Two animals each were sacrificed to check for the study after transplantation of $2 \times 10^6$ tumor cells.
† Expressed as length × width.
‡ Invasion of tumor nodule was seen from submucosa (sm) or mucosa (m) to muscle layer (pm) and adventitia without ($a_0$) or with ($a_1$) infiltration.
§ Lymph node metastases were seen as large as pea size (+), little finger's head size (#) and thumb's head size (###).
‡// Lung metastases was shown as scattered up to miliary size (+), multiple up to rice grain size (#) and conglomerated up to pea size (###).
¶ Average body weight (mean±s.e.) of 3 rabbits followed up to 5 weeks.
** Body weight before inoculation.

The 1st week. Inoculated tumor cells were growing in the submucosal layer with a pattern of irregular solid sheets or nests. Their histology was essentially similar to that of original V$_2$ tumor in the thigh (Fig. 1). No ulceration nor involvement of the mucosal layer was seen. These nests or sheets were surrounded by rather thin fibrous connective tissue with scanty capillary networks. Slight to moderate infiltration of inflammatory cells such as lymphocytes, plasma cells, and eosinophils in and around nodularly growing tumor tissue was observed (Fig. 2). The tumor cells were large ovoid or polygonal with round to oval nuclei. One or more small to medium-sized nucleoli were predominant. Their nuclear size varied slightly. Mitotic figures were frequently found. The nuclear chromation was finely granular without marginal condensation. Cytoplasms stained acidophilic
with granules. The tumor was nodular and rather well-circumscribed without encapsulation and extended to the muscle coat. No metastasis to the regional lymph nodes nor to other distant organs were present.

The 2nd week. There appeared outgrowth of the tumor on the mucosal surface with erosion (Fig. 3). In the center of nodular growth, multiple foci of patchy necroses were seen. The tumors were invading the lower two thirds of the muscle coat. No metastasis was present.

The 3rd week. The tumors grew much larger and extended to the adventitia.
More marked central necrosis of tumor nests was present. Essential characteristics of the arrangement and structure of the tumor cells were well preserved. Apparent metastases to the lymph nodes (Fig. 4) and the lungs (Fig. 5) were disclosed. In the lungs, metastatic nodules with a diameter of up to 1.5 mm were disseminated throughout the parenchyme.

The 4th and 5th weeks. The adventitial layer was more deeply involved by

Fig. 3. Two weeks after inoculation. Mucosal layer was invaded by tumor growth showing erosion.

Fig. 4. Metastatic involvement of the lymph node 3 weeks after inoculation (×100, H.E. stain).
tumors. In some areas, the tumors were partially exposed on the adventitial free surface. Metastatic nodules in the lungs were larger than those of the 3rd week, up to 2.5 mm in diameter at the 4th week, and they showed conglomeration of nodules at the 5th week. In addition to lung metastases, frequent hepatic metastases were noted in the 5th week. No apparent metastases to other sites were detected even in this period. The average body weight of 3 rabbits followed up to 5 weeks after the inoculation suggested that the decrease in body weight reflected the extension of metastases.

**DISCUSSION**

Our present study showed that a malignant tumor was easily induced within a week in the rabbit urinary bladder. The procedure was simple and time-saving with highly reproducible results. The tumors were confined to the submucosal layer for 1 to 2 weeks and then exulcerated into the bladder lumen without any manipulation to the mucosa such as superficial scarification. At the end of 3 weeks metastases were found in the para-aortic lymph nodes and the lungs. Histological examination of the lymph node and lung confirmed the lymphogenous metastases. In spite of the decreasing growth rate of the tumor in the urinary bladder at the end of the 4th week, extensive metastases in the lymph node and the lung were observed at weekly examinations and the decrease in body weight reflected those metastases.

Our tumor model satisfies the criteria for malignant growth, tumor localization, and metastases. Therefore, this malignant bladder tumor of rabbits may prove...
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to be applicable to a variety of experimental researches. One important advantage is the possibility of studying the effectiveness of different methods of regional treatment of urinary bladder cancer, such as transurethral tumor resection, use of laser, local hyperthermia and local chemotherapy (Kato et al. 1979). On the other hand, one important drawback is that this carcinoma is alien from urinary bladder cancer though having the same localization. A further disadvantage is poor vascularization with formation of central necrosis in even non-treated tumor. However, the criteria for the effectiveness of experimental transurethral treatments are primarily the reduction rates of tumor size and metastases, and secondarily the extent and histological nature of necrosis. So, this tumor offers advantage for the study of regional treatment simulating human situation and not for histologic research on the effectiveness of a treatment for malignant tumors.

In comparison with the Harzmann’s model (Harzmann et al. 1979), our transplanted bladder tumor in rabbits has some characteristics. In their model, superficial scarification of the mucosa was performed at the time of inoculation, which lead to exulceration of the tumor into the bladder lumen and the tumor metastases were found mainly in the liver. In our model, the tumor grew into the bladder lumen without any manipulations such as superficial scarification of the mucosa. So, the tumor cell transplantation can be done more speedily and variability in the tumor size is small under different experimental condition. Another characteristic is metastases in the para-aortic lymph nodes and the lungs. This model of lymphogenous metastases from the urinary bladder to the lung many be suitable for studying lymphogenous metastases of human urinary bladder cancer.

Acknowledgments

We appreciate Mrs. E. Nemoto for preparation of the manuscript. This work was supported in part by a Grant-in-Aid for Cancer Research from the Ministry of Health and Welfare, Japan (53–19).

References