A Metastatic Model of Human Colon Cancer Constructed Using Cecal Implantation of Cancer Tissue in Nude Mice

TOSHIHARU FURUKAWA,1 TETSURO KUBOTA,1 MASAKI WATANABE,1 TSONG-HONG KUO,1 HIDEKI NISHIBORI,1 SUGURU KASE,1 YOSHIRO SAIKAWA,1 HIROAKU TANINO,1,2 TATSUO TERAMOTO,1 KYUYA ISHIBI,1
and MASAKI KITAJIMA1

1The Department of Surgery, School of Medicine, Keio University, 35 Shinanomachi, Shinjuku-ku, Tokyo, 160 Japan
2The Department of Thoracic Surgery, Wakayama Medical College, 27 Kyubancho, Wakayama-shi, Wakayama, 640 Japan

Abstract: COL-2-JCK, a human colon cancer xenograft line able to be transplanted into nude mice, was implanted in the subserosal layer of the cecum, either as cancer tissue or as a single cell suspension. When the cancer tissue was used for the cecal implantation, 100% extensive local tumor growth and a high incidence of metastases to the regional lymph nodes, peritoneum, liver, and lung was observed. In contrast, when the cell suspension of this line was injected into the cecal wall, no metastases were observed, with significantly reduced local tumor growth. The use of cancer tissue maintaining the original cancer tissue structure is therefore considered imperative for allowing full expression of the biological characteristics of cancer cells. This nude mouse model using the cecal implantation of cancer tissue should thus prompt further study on the biology of human colon cancer.

Key Words: human colon cancer, metastases, nude mouse

Introduction

Liver metastasis presents a major problem in the treatment of colon cancers. Despite this, very few treatment strategies are effective in the prevention of liver metastases from colon cancers. Thus, appropriate animal models for the metastasis of colon cancer could play an extremely important role in the search for new therapies for colon cancer. Although human tumor xenografts grown subcutaneously in athymic nude mice closely resemble the original tumors morphologically, biologically, and biochemically,1-2 these tumors rarely metastasize.3-7

The recent work of Fidler7 and others8-11 showed that implanting human cancer cells in nude mice to the corresponding organ from which the cancer cells were derived in humans resulted in much higher metastatic rates. For example, human colon cancer cells were disaggregated and injected into the cecal wall of nude mice to produce tumors that eventually metastasized to the liver, demonstrating that cecal implantation can enhance the metastatic capability of human colon cancer cells in nude mice. However, Hoffman and others indicated that human cancer cell suspensions used for implantation in the corresponding organs in nude mice may not express the full metastatic potential of the original tumor compared to implantation using cancer tissue maintaining the original cancer tissue structure.12-13 In this report, we describe the application of this model to human colon cancer, reporting how the implantation of human colon cancer tissue to the subserosal layer of the cecum of nude mice resulted in 100% extensive local growth and a very high incidence of metastases. In contrast, no metastasis was observed, with significantly reduced local tumor growth, in the nude mice inoculated with cell suspensions to the cecal wall.

Materials and Methods

Six- to 8-week-old male Balb/c nu/nu mice weighing 20–22 g, which had been bred in the Central Institute for Experimental Animals, Kawasaki, Japan, were purchased from CLEA Japan (Tokyo, Japan). COL-2-JCK, a human colon cancer xenograft, was kindly provided by Dr. T. Nomura of the Central Institute for Experimental Animals14 and maintained by serial transplantation into nude mice at the Keio University School of Medicine.

Tumors in the exponential growth phase in nude mice were resected aseptically, then necrotic tissues were cut away and the remaining healthy tumor tissues were scissor-minced into pieces about 3-mm in diameter in Hank's balanced salt solution. Each piece of
Table 1. Local tumor growth and metastases observed after the cecal implantation of COL-2-JCK

<table>
<thead>
<tr>
<th>Cecal implantation method</th>
<th>Local tumor growth</th>
<th>Regional lymph nodes</th>
<th>Peritoneum</th>
<th>Liver</th>
<th>Lung</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer tissue</td>
<td>12/12*</td>
<td>6/12</td>
<td>10/12</td>
<td>9/12</td>
<td>6/12</td>
</tr>
<tr>
<td>Cell suspension</td>
<td>3/12</td>
<td>0/12</td>
<td>0/12</td>
<td>0/12</td>
<td>0/12</td>
</tr>
</tbody>
</table>

* Data are expressed as the number of mice in which local tumor growth or metastases were observed/number of mice evaluable

P < 0.01, by the chi-squared test, between the cancer tissue and cell suspension

Tumor was weighed on a Mettler AM 100 balance (Mettler Toledo AG, Switzerland), and adjusted to 50 mg with scissors. Mice were anesthetized with 2.5% Avertin and an incision was made through the left lower abdominal pararectal line and peritoneum. The cecal wall was carefully exposed and part of the serosal membrane, about 2-mm in diameter, was mechanically injured, using scissors. One 50-mg tumor piece was then fixed on each site of injury on the serosal surface with a 4-0 polyglycolic acid (Dexon) transmural suture. The cecum was then replaced in the peritoneal cavity, and the abdominal wall and skin was closed with 4-0 polyglycolic acid (Dexon) sutures. All animals were kept in a sterile environment.

As another approach, tumor pieces were further scissor-minced as finely as possible and incubated at 37°C for 30 min with an enzyme cocktail containing 0.5 mg/ml actinase E (Kaken Pharmaceutical, Tokyo, Japan), 0.2 mg/ml collagenase (type I; Sigma, St. Louis, Miss.), and 0.2 mg/ml DNase I (type IV; Sigma). After incubation, homogenates were passed through a stainless steel mesh (200 μm/s) and the filtrates were washed in RPMI-1640, followed by centrifugation for 10 min at 3,000 rpm, after which the tumor cells were suspended in RPMI-1640 to a cell concentration of 4 × 10⁶ cells/ml. Almost 100% of the tumor cells were shown to be viable by the trypan-blue test. The tumor cell suspension was then injected into the carefully-exposed cecal wall, as described above, at a volume of 0.05 ml (2 × 10⁷ cells) per mouse. Since approximately 500 mg of tumor pieces was necessary to yield 2 × 10⁷ disaggregated tumor cells, the total amount of tumor cells implanted in each mouse as a cell suspension was tenfold that of the cancer tissue.

Mice were sacrificed 12 weeks after implantation or earlier if they developed signs of distress. Actual sacrifices were performed 4–6 weeks after implantation in the case of cancer tissue implantation, and on the 12th week in the case of cell suspension implantation. Autopsies were performed immediately, at which time tumors on the cecal wall were removed, weighed, and examined histologically. The lungs, liver, peritoneum, and lymph nodes in the peritoneal cavity and other organs were processed for the routine histological examination of metastases after careful macroscopic examination.

Results

Table 1 shows the local tumor growth and metastases observed after the cecal implantation of COL-2-JCK as cancer tissue or the cell suspension. Following the cecal implantation of cancer tissue, all 12 of a total 12 mice had local tumor growth (Fig. 1A), 6 of 12 had regional lymph node metastases, 10 of 12 had peritoneal metastases, 9 of 12 had liver metastases (Fig. 1B), and 6 of 12 had lung metastases (Fig. 1C). In contrast, following the cecal implantation of the cell suspension, only 3 of 12 mice had any local tumor growth and none had metastases.

Discussion

Cecal implantation was recently been used for developing rodent models of metastatic human colon cancer. However, the cell lines and disaggregated cells used were obtained from disrupting the original structure of the human tumor tissue, which may possibly have led to a change in the nature and biological behavior of the tumor, and could explain the greatly reduced metastatic rate observed in the present study, compared to the cecal implantation of cancer tissue.

It should be emphasized that almost 100% of the tumor cells in the cell suspensions utilized in the present study were viable as determined by the trypan-blue assay, and that the cell number inoculated into the cecal wall in the cell suspension was 2 × 10⁷ per mouse. This resulted in ten times the amount of tumor cells than in the cancer tissue being sufficient for local tumor formation, as indicated by other studies. Therefore, cell viability and the number of cells in the inoculum of
Following the cell suspension implantation, mice were also sacrificed 4–6 weeks after implantation initially, but no grossly identifiable metastatic nodules were observed. Therefore, we changed the time of sacrifice to 12 weeks after the cell suspension implantation in order to allow sufficient time for the implanted cancer cells to develop metastatic nodules, as indicated in other studies using the cecal implantation of a cell suspension. On the other hand, following the cancer tissue implantation, grossly visible metastases developed within 4–6 weeks, and no mice survived for as long as 12 weeks, due to the extensive spread of the tumors. Thus, no local tumors or metastases disappeared naturally in this study. Furthermore, it is possible that interstitial lymphocytes contained in the cancer tissue implanted in the cecum modified the metastatic behavior, although this condition was thought to the essentially identical to the implantation of dissociated cancer cells in which the interstitial cells were not eliminated.

Although the tumor lines used in this study are reported to metastasize to the liver and the peritoneum in nude mice after intrasplenic or intraperitoneal injections of disaggregated cell suspensions, these models without local tumor formation could not reflect the natural course of metastases. For example, intrasplenically-injected cells were reported to be identified in the liver after 5 min. The model of cecal implantation includes local invasive tumor growth, which is an important mechanism of metastases, but this could not be evaluated in the other models. This metastatic model constructed using the cecal implantation of cancer tissue from human colon cancers should thus prompt further studies on the biological behavior of human colon cancer.

References