Minimal liver resection strongly stimulates the growth of human colon cancer in the liver of nude mice

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Abstract

Partial hepatectomy has been widely employed in clinical practice as the therapy of choice for primary and metastatic liver tumors. However, the recurrence rate after the treatment remains high, which is most likely due to the growth of residual microscopic lesions. Previous studies in murine models demonstrated that a 70% hepatectomy significantly accelerated the growth of ectopically implanted tumors. In this study, we reported the effect of partial hepatectomy on the growth of two human colon cancers (Co-3 and AC3603) implanted in the liver of nude mice using the technique of surgical implantation of histologically intact tumor tissue. Our results showed a dramatic acceleration of tumor growth following 30% partial hepatectomy, which resembles clinical procedures. Tumor volumes were assessed with calipers on day-15 by abdominal palpation and on day-30 at autopsy by direct measurement. For both Co-3 and AC3603, tumor volumes in the hepatectomized animals were significantly larger than the control at the above two time points (P < 0.001). The results demonstrate the stimulating effect of partial hepatectomy directly on the tumor growth in the liver, in contrast to previous studies on ectopic tumors. Furthermore, since conservative partial hepatectomy (30%) is normally used in clinical practice for surgical treatment of liver metastasis, the animal models presented here should be useful for the clinical investigation of the high recurrence rate of liver metastasis following partial hepatectomy.

Introduction

Each year 150,000 new cases of colorectal cancer are diagnosed in the United States [1]. More than 50% of these patients develop liver metastasis [2]. For patients with liver metastasis from colon cancer, the only curative treatment currently available is partial hepatectomy. However, the recurrence rate following partial hepatectomy is high with approximately half of patients developing recurrence in the remnant liver as well as in other organs [3]. The cause is believed to be mostly due to the growth of residual microscopic metastatic lesions within the residual liver.

Several laboratories have investigated the influence of partial hepatectomy on the growth of implanted tumors in animal models. Most of the investigators claimed that partial hepatectomy had a stimulating effect on tumor growth [4–6]. They documented that the stimulating effect could only be seen following 70% partial hepatectomy and a more conservative liver resection (30%) could not achieve such an effect. In these studies tumors were implanted at sites other than the liver, such as the subcutis.

In the present study we demonstrate that a 30% hepatectomy is sufficient to significantly stimulate the growth of human colon cancer surgically implanted on the nude mice liver. The model in this study closely resembles the stimulation of human colon cancer in the liver after clinical partial hepatectomy.

Materials and methods

Animals

Athymic nu/nu Balb/c mice (Charles River Laboratories, Wilmington, Massachusetts) of both sexes, 6–7 weeks old, were used in the study. They were maintained in a specific pathogen-free environment in compliance with USPHS guidelines governing the care and maintenance of experimental animals. All animal studies were conducted in accordance with the principles and procedures outlined in the National Institute of Health Guide for the Care and Use of Animals under the assurance number A3873-1. Mice were fed with autoclaved laboratory rodent diet (Teeklad LM-485, Western Research Products, Orange, California).
Human colon cancer

Two human colon cancers were used in this study. Cell line Co-3 was kindly provided by Dr Testuro Kubota of Keio University, Tokyo, Japan. It was a well-differentiated adenocarcinoma of the colon and was derived from a metastatic lesion in the lung of a 39-year-old female patient in 1979. AC3603 was also a well-differentiated adenocarcinoma. It was a patient specimen obtained from a 48-year-old female who underwent transverse colon resection at UCSD Medical Center, San Diego, California. The tumor tissue was initially implanted subcutaneously in nude mice. Tumor fragments used in this study were from the 4th passage.

Partial hepatectomy

Mice were anesthetized with isoflurane inhalation and put in a supine position. The abdomen was sterilized with iodine and alcohol swabs. A left subcostal incision was made to expose the liver. The left lobe of the liver was identified and its bilio-vascular bundle was ligated with a 6-0 nylon suture. Then the entire left lobe was resected. The resected lobe was approximately 30% of the total liver.

Tumor implantation on the nude mice liver

Tumor tissue fragments were implanted on the middle lobe of the liver, as described below, immediately after partial hepatectomy.

Co-3 and AC3603 tumor tissues were harvested from subcutaneously-growing stock in nude mice. They were carefully inspected under a dissecting microscope (5×) to remove necrotic tissue. Then the collected tumor tissues were equally divided into small pieces of 1 mm³ each. The middle lobe of the liver of the nude mice was located through the left subcostal incision. A small cut on the glissonian capsule of the middle lobe was then performed. Two pieces of the above tumor tissue fragments were inserted into the incision on the liver and then sutured in place using an 8-0 nylon suture. The abdomen was closed with a 6-0 silk suture.

Fourteen mice were used for each Co-3 and AC3603 with a total of 28 mice for the entire study. They were equally divided into two groups: one with 30% partial hepatectomy and one without hepatectomy. Tumor tissue fragments were mixed thoroughly before the implantation procedure began to ensure each mouse received equally viable tissues.

Evaluation of tumor growth

On day-15 after implantation, tumor sizes were assessed through abdominal wall palpation with caliper measurement. At day-30 all mice were sacrificed and tumor width and length were measured directly. The formula ‘width’ × length × 0.5” was used to estimate tumor volume [20, 21].

Statistical analysis

The tumor volume was analyzed using the Student’s t-test.

Results

For both the Co-3 and AC3603 colon tumors, a dramatic difference in tumor size was observed between the 30% hepatectomy group (Figure 1A) and the intact-liver group (Figure 1B). Co-3 and AC3603 grew much faster in the nude mice liver after 30% hepatectomy. Tumor size was estimated by palpation through the abdominal wall with caliper measurement at day-15 after tumor implantation (Figure 2). In the mice with 30% partial hepatectomy, the mean tumor volumes were 1185 mm³ for Co-3 and 784 mm³ for AC3603, while in those without partial hepatectomy the tumor volumes were 191 mm³ and 60 mm³, respectively (P < 0.001). On day-30 after tumor implantation all mice were sacrificed. At this time, the mean tumor volumes in the partial hepatectomy groups were 7369 mm³ for Co-3 and 6229 mm³ for AC3603. In the intact liver groups, the mean tumor volumes were 1060 mm³ for Co-3 and 575 mm³ for AC3603 (Figure 2). Tumors were removed and then weighed using an electronic balance. On day-30, the mean tumor weights in the partial hepatectomy groups were 3919 mg for Co-3 and 1779 mg for AC3603. In the intact liver groups, the mean tumor weights were 306 mg for Co-3 and 218 mg for AC3603 (Table 1). These differences in tumor volume and weight in the hepatectomized animals compared to control for both tumors was highly statistically significant (P < 0.001) (Table 1). Two mice in the Co-3 hepatotectomy were one in the AC3603 hepatotectomy group that showed multiple liver metastases. None in the control groups were found to have diffuse liver metastasis.

Discussion

Recurrence after liver resection for metastatic colon cancer poses a major treatment problem. Approximately 40% of patients who undergo liver resection for colon cancer liver metastasis have recurrences in the liver [3].

The effect of partial hepatectomy on tumor growth has been controversial. Castillo and Doerr reported the beneficial effect of partial hepatectomy on survival of mice with liver metastases from ileocolic vein injection of cell suspensions of a mouse colon adenocarcinoma [7,8]. In another study, Minoro et al [9] demonstrated after extensive partial hepatectomy (70%) complete regression of a subcutaneously growing mouse hepatoma from a cell suspension injection. On the other hand, most of the investigators demonstrated that partial hepatectomy enhanced tumor growth in experimental animals. Gutman et al. [5] reported an acceleration of human colon tumor growth in the nude mice subcutis after partial liver resection. Mituzutani et al. [6] reported that partial hepatectomy promoted hepatic tumor growth after portal vein injection of hepatoma cell suspensions.

According to Gutman et al., tumor implantation and growth could be influenced by the host immune status, and partial hepatectomy could modulate host immune responses to the inoculated tumor cells. Oshima et al. [10] and Nakagawa et al. [11] reported that OK-432, a therapeutic compound derived from a Streptococcal preparation
Table 1. Comparison of final tumor weights of human colon cancer Co-3 and AC3603 implanted on the intact and partially resected (30%) liver of nude mice.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Co-3 tumor weight (mg)</th>
<th>AC3603 tumor weight (mg)</th>
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<tbody>
<tr>
<td>Intact liver</td>
<td>306 ± 112</td>
<td>218 ± 53</td>
</tr>
<tr>
<td>Partial hepatectomy (30%)</td>
<td>3919 ± 1914&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1779 ± 862&lt;sup&gt;b&lt;/sup&gt;</td>
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<sup>a</sup> P < 0.001.
<sup>b</sup> P < 0.001.

Figure 1. (A) Human colon cancer Co-3 growing in the nude mouse liver after partial hepatectomy (30%) (arrows). (B) Human colon cancer Co-3 growing in the nude mouse liver without partial hepatectomy (30%) (arrow).

Figure 2. Human colon cancer Co-3 and AC3603 were implanted on the liver of nude mice using the surgical implantation technique. Partial hepatectomy (30%) was performed in one arm. As can be seen in the figure, partial hepatectomy significantly stimulated tumor growth on the liver (P < 0.01). Tumor measurement was performed on day-15 and day-30 after tumor implantation.
which stimulates mouse natural killer activity, stimulated host defenses against tumor cells in hepatectomized mice. However, Mitziutani et al. [6] found that OK-432 did not reduce the incidence or the number of artificially induced liver metastases. Mitziutani et al. [6] and Nakamura et al. [12] both suggested that the release of growth factors by hepatocytes and other cells may be responsible for tumor growth after hepatectomy in murine models. The release of growth factors was considered to be related to the extent of liver resection.

In this study we used surgical techniques to implant intact tissue fragments of human colon cancer directly on the nude mice liver following partial hepatectomy. Previous studies on the enhancement of tumor growth after partial hepatectomy demonstrated an effect only after a 70% partial hepatectomy. In these previous studies tumor cell suspensions were used and they were injected into sites other than the liver. In contrast, the present study demonstrated a strong enhancement of tumor growth in the liver following a 30% partial hepatectomy.

In this study, the human colon cancer tissue fragments, were directly implanted on the nude mice liver to mimic clinical liver metastasis from colon cancer. Although hepatic implantation does not exactly duplicate the clinical event of liver metastasis from colon cancer, it serves as a useful method to observe the effect of local growth factors induced by partial hepatectomy on the growth of the colon tumor in the liver. The technique of using histologically-intact tumor tissue for transplantation in nude mice was based on the idea that the 3-dimensional tumor-stromal tissue architecture in a tumor mass plays a role in growth and progression [13-19]. The supportive stromal cells maintain the three-dimensional architecture of the tumor and allow growth factors, angiogenic factors and other stimulating factors to interact more efficiently between the tumor cells [15-19]. Tumor cell suspensions lack 3-dimensional stromal architecture. Apparently, the three dimensional structure of the implanted tumor tissue in this study aided in the growth stimulation of the tumor despite a conservative liver resection (30%).

Local growth factors in the liver seem to be responsible for the stimulation of tumor growth after liver resection. The 30% partial hepatectomy, a conservative liver resection, resembles clinical protocols for treatment of colon cancer liver metastasis more than the highly aggressive two-thirds liver resection that is not clinically relevant [3]. The animal model reported here should be useful for investigating the stimulating effect of partial hepatectomy on the growth of metastatic liver cancer.

Future experiments will use an orthotopic human colon cancer nude mouse model with a metastatic propensity to the liver to test if systemic factors contribute to the increased growth of the colorectal tumor on the colon.

Future experiments will be carried out to determine if a 30% partial hepatectomy and 70% hepatectomy exert differential growth effects on local and metastatic tumors.

References