"Seed" to "Soil" is a Return Trip in Metastasis

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Abstract. A critical aspect in understanding and treating cancer progression and metastasis is the relationship of the host originating organ and metastatic "soil" organs that support the growth and progression of the cancer "seed". We have recently demonstrated that there is a great difference in seemingly similar visceral organs, the colon and the stomach to support the growth progression of transplanted human colon tumors in nude mice. To further understand the relationship of seed and soil in cancer, we transplanted the metastatic human colon tumor CO-3 on the liver of nude mice, which is a usual metastatic soil organ for this tumor if transplanted to the nude-mouse colon. The intrahepatically-transplanted CO-3 tumor grew extensively on the nude-mouse liver without intra-hepatic metastasis. However, cecal growth, peritoneal dissemination, and invasiveness were noted after extensive growth on the liver with no spread to other organs. This phenomenon suggested that the intra-hepatically transplanted tumor could "reversibly metastasize" to the orthotopic site and secondarily spread into the abdominal cavity. The observation reported here suggests that "seed" to "soil" is reversible in metastasis in that the tumor can spread in either direction between two "matched" organ "soil".

In 1889, Paget analysed 735 autopsy records of women with breast cancer. The non-random pattern of visceral metastasis suggested to him that certain tumor cells (the "seed") had a specific affinity for the milieu of certain organs (the "soil"). Paget suggested that metastases resulted only when the seed and soil were matched. In this paper we report that, indeed, "seed and soil" are matched with regard to a human colon tumor transplanted orthotopically (1-4) or intra-hepatically in nude mice.

A critical aspect in understanding and treating cancer progression and metastasis is the relationship of the host originating organ and metastatic "soil" organs that support the growth and progression of the cancer "seed." We have recently demonstrated that there is a great difference in seemingly similar visceral organs, the colon and the stomach, to support the growth and progression of transplanted colon tumors in nude mice (7). To understand further the relationship of seed and soil in cancer, we transplanted the metastatic human colon tumor Co-3 on the liver of nude mice, which is a usual metastatic soil organ for this tumor if transplanted to the nude-mouse colon (2-4). The intra-hepatically-transplanted Co-3 tumor grew extensively on the nude mouse liver without intra-hepatic metastasis. However, cecal growth, peritoneal dissemination and invasiveness were noted after extensive growth on the liver with no spread to other organs. This phenomenon suggested that the intra-hepatically transplanted tumor could "reversibly metastasize" to the orthotopic site and secondarily spread into the abdominal cavity. The observation reported here suggests that "seed" to "soil" is reversible in metastasis, in that the tumor can spread in either direction between its two "matched" organ "soils."

Materials and Methods

Mice. Four-week-old 
m/mu mice of both sexes were used for tumor implantation. All animals were maintained in a sterile environment; cages, bedding, food and water were all autoclaved. All animals were maintained on a daily 12-hr light/12-hr dark cycle. Bethprin pedi-

atric suspension (containing sulfaflaxamide and trimethoprim) was added to the drinking water. NIH guidelines were followed for all animal experimentation.

Human colon cancer xenografts. The human colon cancer xenograft Co-3, a well differentiated adenocarcinoma (4), was used in this study.


Key Words: Metastasis, intrahepatic tumor transplantation, human colon cancer xenografts.
Intrahepatic tumor tissue implantation. Subcutaneously growing tumors at the exponential phase in nude mice were resected aseptically, necrotic tissues were cut away, and the remaining healthy tumor tissues were scissor minced into pieces about 1-2 mm³ size in Hanks' balanced salt solution (2, 3).

Nude mice were anesthetized with isoflurane (Forane) inhalation. An incision was made through the left upper abdominal pararectal line and peritoneum. The left lobe of the liver was carefully exposed, and the liver was cut about 3 mm with scissors. Two to three pieces of 1-2 mm³ size were put on the nude mouse liver and attached immediately with double sutures using 8-0 nylon with an atraumatic needle. After confirmation that no bleeding was occurring, the liver was then returned to the peritoneal cavity. The abdominal wall and skin were then closed with 6-0 black silk sutures.

Evaluation of growth and metastasis of intrahepatically implanted tumors. When the palpable tumor in the liver reached about one centimeter, the animals were sacrificed and autopsied. Gross tumor growth was observed and all major organs were fixed in formalin and prepared for sectioning and staining with hematoxylin and eosin by standard procedures.

Results and Discussion

In all three mice with the Co-3 human colon tumor tissue implanted on their liver, the tumor grew into a large intrahepatic mass (Figure 1, Table I). The liver masses ranged in size from 10 x 9 mm to 20 x 17 mm. Most interestingly, after 49 days growth, as seen on a gross scale in Figure 2, the intrahepatically growing human colon tumors metastasized to the cecum in two out of the two animals allowed to live to this point, and in addition in one animal the tumor further metastasized to the peritoneal wall. Microscopic findings of hematoxylin-and eosin-stained sections also revealed that the intrahepatically-growing tumor reversely metastasized to the serosal layers of the cecum (Figure 3).

Orthotopically-transplanted Co-3 human colon tumor tissue grows and invades the muscularis propria, submucosa, mucosa and the lymphatic ducts of nude mice (2-4). Lymph node metastasis and liver metastasis also result (2-4). In contrast, Co-3 tumor heterotopically transplanted on the nude mouse stomach grows expansively without invasion of the submucosal, mucosal layer and regional lymph node metastases. We observed here that the intrahepatically transplanted tumor reversely metastasizes to the orthotopic site and secondarily spread into the abdominal cavity of the mice that grew the tumor for 49 days. Interestingly, the mouse that grew the tumor for only 25 days apparently had insufficient time for

![Figure 1. Growth of intrahepatically transplanted human colon adenocarcinoma Co-3 viewed at the gross level in nude mice. A and B are two views of typical intrahepatically-growing Co-3. C is a cut section of B. The Co-3 tumor was transplanted intrahepatically as described in the text.](image)

<table>
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<tr>
<th>Mouse No.</th>
<th>Tumor</th>
<th>Days after operation</th>
<th>Size (mm³) of intrahepatic colon tumor</th>
<th>Intrahepatic metastasis</th>
<th>Reverse metastasis to cecum</th>
<th>Peritoneal dissemination</th>
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<tbody>
<tr>
<td>(1)</td>
<td>Co-3</td>
<td>25</td>
<td>10 x 9</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(2)</td>
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<td>10 x 11</td>
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<td>+</td>
<td>-</td>
</tr>
<tr>
<td>(3)</td>
<td>Co-3</td>
<td>49</td>
<td>20 x 17</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

The human colon adenocarcinoma Co-3 was transplanted intrahepatically in nude mice as described in the text. After 25-49 days, the animals were sacrificed and explored in autopsies for gross tumor formation.

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Figure 2. Gross view of "reverse metastases" of intrahepatically transplanted human colon adenocarcinoma Co-3 in nude mice. A and B intrahepatic growth (open arrow) and cecal reverse metastasis of human colon tumor Co-3 in nude mice (black arrow). See legend to Figure 1 and text.

Figure 3. Microscopic view of "reverse metastases" of intrahepatically-transplanted human colon adenocarcinoma Co-3 in nude mice. A) Intrahepatic growth of human colon tumor Co-3 (arrow). B) Serosal reverse metastases of Co-3 (arrow). See legend of Figure 1 and text for details.
reverse metastases (Table I, Figure 1-3). Our results obtained with orthotopic and intrahepatic transplantation of a human colon tumor in nude mice thus confirm Paget’s hypothesis, in that the originating and metastatic organs are matched soils for the cancer seed which seemingly does not involve other organs. The results demonstrate the power of relevant transplant models in rodents to probe the mechanisms of metastasis. The common features of the matched organs of cancer spread need to be investigated further in order to understand metastasis (5, 6).

References


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