Extensive Liver Metastasis from Human Colon Cancer in Nude and Scid Mice after Orthotopic Onplantation of Histologically-Intact Human Colon Carcinoma Tissue

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Abstract. Clinically-relevant animal models of human cancer are greatly needed for the study of human cancer biology and the development of new cancer therapeutics and diagnostics. We report here that by orthotopically transplanting histologically-intact human colon cancer to the colon of the immuno-deficient nude and scid mouse mutants that extensive local growth and liver metastases occur consistently even after extensive in vivo orthotopic passage. We demonstrate that the liver metastases arise by hematogenous spread. The models described in this report for human colon cancer should prove useful for individual cancer patients as well as for basic and applied studies to develop improved treatment.

The development of better animal models for human cancer are important for improved treatment for this disease. Subcutaneous or intra-muscular xenografts of immunodeficient mice have low or non-existent metastatic capability even tumors that were highly metastatic in the patients from whom the tissue were derived (1-5). A number of studies have indicated that implanting human tumor cells orthotopically in the corresponding organ of nude-mice resulted in much higher metastatic rates. For example, human colon cancer cells, when dissociated, grown in culture, and subsequently injected into the cecum of nude mice can produce tumors that eventually metastasize to the liver (5-9). Similar results also have been achieved for orthotopic implantation of cell lines of other cancers (5).

Our approach is to avoid disruption of tissue integrity and to orthotopically implant histologically-intact tumor tissue directly. With this overall strategy, we have constructed models of human colon cancer in nude mice (10) and human bladder cancer in nude mice (11, 12), human pancreatic cancer in nude mice (13) and human lung cancer in nude and severe combined immunodeficient (scid) mice (14) that demonstrate the variety of clinical behavior that occurs in human subjects. We report here that this approach can allow the development of very extensive liver metastases in nude and scid mice after transplantation of human colon cancer tissue to the mouse colon.

Materials and Methods

Four-week-old outbred nu/nu mice and scid mice of both sexes were used for tumor transplantation. All animals were maintained in a sterile environment. Cages, bedding, food and water were autoclaved. All animals were maintained on a daily 12-hour light/12-hour dark cycle. Bactrim Pediatric Suspension (containing sulfamethoxazole and trimethoprim) was added to the drinking water.

Specimens were then inspected, and grossly necrotic and suspected necrotic tissue was removed. Each specimen was equally divided into four to six separated parts, and each part was subsequently cut into small pieces of about 1 mm3. Tumor pieces for each transplantation were taken from each of the four to six parts of the specimen equally. In this way, the chance for viable tissue to be transplanted was maximized.

For transplantation, nude mice were anesthetized, and the abdomen was sterilized with iodine and alcohol swabs. A small midline incision was made and the cecal part of the intestine was exteriorized. Serosa of the site where tumor pieces were to be transplanted was removed. Eight to 15 pieces of 1-mm3 size tumor were implanted on the top of the animal intestine; an 8.0 surgical suture was used to penetrate these small tumor pieces and suture them on the wall of the intestine. The intestine was returned to the abdominal cavity, and the abdominal wall was closed with 7-0 surgical sutures. Animals were kept in a sterile environment.

Results and Discussion

As can be seen in Table 1 and Figure 1, in three of five nude mice in which the human colon tumor Co-3 xenograft line was transplanted as intact tissue into the mouse colon, liver metastases have resulted, in some cases larger than a centimeter (Figure 1A). Figure 1B demonstrates the adenocarcinoma histopathology of the human colon-tumor metastases growing into the nude-mouse liver. Figure 1C also shows the histopathology of the human colon tumor metastases growing in the nude mouse liver after seven passages, demonstrating the stability during passage of the tumor. This same line, when transplanted to the scid mouse colon, also resulted in liver metastases in four of five mice. Figure 1D shows the histo-
Figure 1. Liver metastases of human colonic tumors in immunodeficient mice after orthotopic onplantation.

A. Very large liver metastasis in nude mouse of human colonic tumor Co-3 after orthotopic onplantation to mouse colon (arrow).
B. Histopathology of human colon tumor Co-3 metastasis in nude-mouse liver after orthotopic onplantation.
C. Histopathology of human colon tumor Co-3 metastasis in nude-mouse liver after seven passages and orthotopic onplantation.
D. Histopathology of human colon tumor Co-3 metastasis in scid-mouse liver after orthotopic onplantation.
E. Histopathology of liver metastases of human colon tumor #1594 after orthotopic onplantation to nude mouse colon.
F. Same as E. Please note isolated micrometastasis of human colonic tumor #1594 in nude-mouse liver.
G. Same as E. Please note isolated metastasis of human colonic tumor #1594 in nude-mouse liver blood vessel indicating hematogenous spread.
H. Histopathology of human colon tumor #1594 metastasis in nude-mouse liver after second passage and orthotopic onplantation.
Table 1. Growth and liver metastasis of human colon Co-3 tumor after orthotopic implantation of intact tissue.

<table>
<thead>
<tr>
<th>Mouse type</th>
<th>Number of mice transplanted</th>
<th>Number of mice with local tumor growth</th>
<th>Number of mice with liver metastasis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nude</td>
<td>5</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Scid</td>
<td>5</td>
<td>5</td>
<td>4</td>
</tr>
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pathology of the human colon tumor metastasis in the scid-mouse liver, again demonstrating the adenocarcinoma nature of the tumor. Figure 1E shows a micrometastasis of the poorly-differentiated adenocarcinoma of the human colon cancer from patient #1594 in the liver of the nude mouse. Figure 1F shows an isolated micrometastasis of the human colon cancer in the nude-mouse liver. Note in Figure 1G the human colon tumor in a vessel entering the nude mouse liver, demonstrating the hematogenous spread of the human tumor in the nude mouse. Figure 1 H demonstrates a metastasis of the human colon tumor in the nude mouse liver two passages later, demonstrating stability of the tumor during passage. Thus, the results presented here indicate that the onplant method of orthotopic transplantation can result in very extensive repeatable metastatic behavior of particular colon tumors in immunodeficient mice such as nude and scid. This system should prove very useful for the study of human tumor biology, in particular metastasis, and the evaluation of potential anti-metastatic agents.

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References
1 Sordat B, Fritsche R, Mach JP, Carrel S, Ozzello L, Cerotini, JC:

12 Fu X and Hoffman, RM: Human RT-4 bladder carcinoma is highly metastatic in nude mice and comparable to R1H-transformed RT-4 when orthotopically onplanted as histologically-intact tissue. Int J Cancer in press.

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