Metastatic Behavior of Tumor Cells Isolated from Primary and Metastatic Human Colorectal Carcinomas Implanted into Different Sites in Nude Mice

Raffaella Giavazzi, Debora E. Campbell, J. M. Jessup, Karen Cleary, and Isaiah J. Fidler

Introduction

Most of the data on the metastatic behavior of neoplasms have been derived from studies in rodent systems. Little comparable data exist on the metastatic nature of human cancers. This is due, in part, to the lack of suitable laboratory models for in vivo studies of human cancer metastasis. The availability of the athymic (T-cell-deficient) nude mouse for studies of hetero- and allografting of tissues (1-4) has provided a most valuable tool for examining many aspects of the biological behavior of human tumors in an in vivo situation. In general, human neoplasms growing in nude mice can be shown to closely resemble the original tumor in histopathological, cytological, and biochemical characteristics (4-7). Therefore, the behavior of human tumors in an athymic nude mouse has recently increased (8-16), although the frequency of metastases and the stability have been controversial.

In the present study, we investigated the metastatic behavior, in nude mice, of HCC tumor lines established from primary neoplasms and from metastases. To determine the suitability of the nude mouse as a model for studies of HCC metastasis, the human tumor cells from primary neoplasms and from metastases were injected into different sites. The data demonstrate that both the properties of the HCC cell lines and the host organ environment of the nude mouse accurately reflect metastatic behavior.

Materials and Methods

Animals

Six- to 8-week-old athymic BALB/c nude mice were obtained from the Animal Production Area of the NCI-Frederick Cancer Research Facility. Mice were age and sex matched for each experiment. Throughout the experiments, mice were maintained in a laminar flow cabinet under specific-pathogen-free conditions.

Clinical Characteristics of the Colorectal Carcinoma Patients

Surgical specimens were obtained from 8 patients with primary or metastatic disease. The patients had a median age of 59 years (range, 42-74 years). There were no significant differences in age between the 4 men and 4 women. Six of the 8 patients had primary rectal carcinomas. Tumors HCC-M2193 and HCC-M14328 were primary tumors located at the sigmoid. Six of 8 patients had elevated carcinoembryonic antigen levels before operation, including 3 without evidence of metastatic disease. The patients had a median age of 59 years (range, 42-74 years). There were no significant differences in age between the 4 men and 4 women. Six of the 8 patients had primary rectal carcinomas. Tumors HCC-M2193 and HCC-M14328 were primary tumors located at the sigmoid. Six of 8 patients had elevated carcinoembryonic antigen levels before operation, including 3 without evidence of metastases (Table 1).

Establishment of Tumor Lines

Tumor specimens from primary HCC or from HCC metastases were obtained immediately after surgery and processed according to published techniques (24, 25). Briefly, tumor tissue was dissected free of necrotic areas, connective tissue, and blood clots and rinsed three times in cold (4°C) Eagle's minimum essential medium containing 1% human serum albumin (Travenol Laboratories, Glendale, CA) and antibiotics (penicillin, 100 units/ml; streptomycin, 100 μg/ml; amphotericin B, 3 μg/ml; and gentamicin, 50 μg/ml, all purchased from Gibco (Grand Island, NY)). The tissue was then cut into 1-3-mm³ fragments using a sterile scalpel blade. The fragments were subjected to a sequential enzymatic digestion of 20 min at 37°C in Eagle's minimum essential medium containing collagenase type I (200 units/ml) and DNase (270 units/ml) (Sigma, St. Louis, MO). To complete the dissociation process, hyaluronidase type IV (35 NF units/ml; Sigma) was added to the enzyme mixture. After enzymatic dissociation, the cells were maintained at 4°C. The cell suspension was filtered through a 4-layer sterile...
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Table 1 Clinical characteristics of patients with primary or metastatic colorectal carcinomas

<table>
<thead>
<tr>
<th>Tumor line</th>
<th>Duke's classification</th>
<th>Tumor source</th>
<th>Blood group</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>CEA* (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCC-P4733</td>
<td>B₁</td>
<td>Rectal primary</td>
<td>O+</td>
<td>51</td>
<td>F</td>
<td>3.5</td>
</tr>
<tr>
<td>HCC-P10055</td>
<td>B₁</td>
<td>Rectal primary</td>
<td>B+</td>
<td>74</td>
<td>M</td>
<td>30.4</td>
</tr>
<tr>
<td>HCC-P4491</td>
<td>B₂</td>
<td>Rectal primary</td>
<td>O+</td>
<td>71</td>
<td>M</td>
<td>8.2</td>
</tr>
<tr>
<td>HCC-P8589</td>
<td>B₃</td>
<td>Rectal primary</td>
<td>O</td>
<td>42</td>
<td>F</td>
<td>9.6</td>
</tr>
<tr>
<td>HCC-M14328</td>
<td>C₂</td>
<td>Mesenteric node metastasis</td>
<td>A+</td>
<td>68</td>
<td>F</td>
<td>1.8</td>
</tr>
<tr>
<td>HCC-M2193</td>
<td>D</td>
<td>Liver metastasis</td>
<td>A+</td>
<td>64</td>
<td>M</td>
<td>8.6</td>
</tr>
<tr>
<td>HCC-M1544</td>
<td>D</td>
<td>Liver metastasis</td>
<td>A+</td>
<td>54</td>
<td>F</td>
<td>172.4</td>
</tr>
<tr>
<td>HCC-M1410</td>
<td>D</td>
<td>Liver metastasis</td>
<td>O</td>
<td>44</td>
<td>M</td>
<td>173.9</td>
</tr>
</tbody>
</table>

* Carcinoembryonic antigen (CEA) was measured by enzyme immunoassay of serum with 3.5 ng/ml the upper limit of normal.

gauze, washed 3 times in serum-free medium, and resuspended in HBSS. For in vivo implantation 2–4 × 10⁶ tumor cells, shown viable by trypan blue exclusion, in a 0.1-ml volume were injected i.m. in the hind thigh. The HCC-M1544 tumor line was the exception, since it was established by s.c. injection of 1 × 10⁶ cells in the flank.

Transplantation Techniques

Tumors were maintained by i.m. transplantations of tumor cell suspension (4 × 10⁶ cells in 0.1 ml solution) prepared from the solid tumors as described above. The data on metastatic capacity of HCC lines were produced with tumor cells obtained from the 3 to 6 transplants in nude mice.

The experiments examined the growth and metastatic potential of cells subsequent to injection into multiple organ sites. Because the enzymatic dissociation of solid tumors may introduce variabilities in the cell suspension preparation, we always used a group of mice given s.c. injections as controls. This practice allowed for comparisons among multiple experiments. Only experiments in which the s.c. injection of human tumor cells yielded a positive result were considered in our study.

The diameters of tumors growing s.c. were measured in centimeters once a week by caliper and the tumor weight was calculated by the formula

\[ \text{wt (g)} = \frac{\text{Length} \times \text{width}^2}{2} \]

Estimates of tumor weight were plotted versus days after inoculation, and the tumor-doubling time was calculated from the resulting graph.

All growing tumors were examined for and found free of reovirus type 3, pneumonia virus of mice, K-virus, Theliler’s virus, Sendai virus, minute virus of mice, mouse adenovirus, mouse hepatitis virus, lymphocytic choriomeningitis virus, ectromelia virus, and lactate dehydrogenase virus in assays performed by Microbiological Associates (Bethesda, MD).

To certify the human origin of the tumors, we harvested representative samples of growing tumors, established them in culture, examined their karyotype, and performed isoenzyme analysis (Authentikit; Cornell Medical, Cornning, NY).

Metastatic Assays

Spontaneous Metastasis Formation. The ability to produce visceral metastases by tumors growing s.c. was examined in groups of mice given injections of 1, 2, and 4 × 10⁶ viable tumor cells suspended in 0.2 ml of HBSS. All injections were delivered into the anterior region of the abdominal flank. Separate groups of mice were given i.m. injections, into the hind thigh, of 4 × 10⁶ viable tumor cells in 0.1 ml HBSS. Once the tumors reached 1.5–2.0 cm in diameter, the leg with the tumor was amputated at the midfemur.

Mice given s.c. injections of tumor cells were killed when the tumors exceeded 2.0–3.0 cm in diameter. Mice given i.m. injections (tumors excised) were killed 6 months after the injection of the tumor cells or when moribund. All mice were autopsied. Lungs, enlarged lymph nodes, and all other organs suspected of containing metastases were fixed in 10% buffered formalin and processed for histological examination.

Experimental Lung Colony Assay. To produce pulmonary tumor colonies (experimental metastases), mice were given i.v. injections, into the lateral tail vein, of 1 × 10⁶ viable cells in a single-cell suspension in 0.2 ml of HBSS. At autopsy, 90 days after the i.v. injection, or when mice were moribund, the lungs were removed and fixed in Bouin’s solution, and the number of lung tumor colonies was determined under a dissection microscope.

Liver Metastasis Assay. The capacity to form tumors in the liver was studied following i.s. injection as described in detail previously (23). Briefly, mice were anesthetized with methoxyflurane and their left flanks were prepared for surgery. A small, cutaneous incision was made in the left flank and carried down through the peritoneal wall. The spleen was carefully exposed and 2 × 10⁶ viable tumor cells in 0.05 ml HBSS were injected under the spleen capsule via a 27-gauge needle.

A visible “paling” of the spleen and the lack of bleeding were the criteria for a successful inoculation. The spleen was then returned into the peritoneal cavity, the abdominal wall was closed with sutures, and the skin was closed with metal wound clips. The mice were killed at various times after injection as indicated and autopsied. Specimens from the lungs, livers, and spleens, as well as lymph nodes suspected to be involved with tumors, were fixed in 10% buffered formalin and processed for histological examinations. At the time of autopsy, liver tumor growths were evaluated in terms of size and number of lesions. These determinations allowed us to develop the following grading of tumor burden in individual livers: 0, no tumor deposits, tumor free; I, histological evidence of tumor growth; II, <10 tumor foci, <1–2 mm in diameter; III, 10–100 tumor foci, <3–5 mm in diameter; IV >100 tumor foci, >5 mm in diameter.

Histopathological Examinations. All specimens used in our studies were classified by routine histopathological examination in the Pathology Department, University of Texas M. D. Anderson Hospital. Xenografts of tumor tissue and organs involved in the metastasis assays were fixed in 10% phosphate-buffered formalin, embedded in paraffin, sectioned at 4-μm intervals, and stained with hematoxylin and eosin.

RESULTS

Tumorigenicity in Nude Mice. Cells isolated from 4 primary tumors, 3 hepatic metastases, and 1 lymph node metastasis of patients with colorectal carcinomas were implanted into nude mice. Subsequent to repeated transfer, continuous tumor lines were established. The tumor-doubling time at a comparable transplant generation (5–6 passages into nude mice) ranged from 3.5 to 8.2 days (Table 2). In general, the 4 tumor lines derived from HCC metastases produced faster growing tumors (average doubling time, 4.1 days) than those produced by cells derived from primary HCC (average tumor-doubling time, 6.6 days).

Histopathological Examinations. The xenografts of the tumors displayed little morphological change from the original tumors in the human (Fig. 1). Occasionally, these neoplasms became more or less differentiated, but the alterations were slight. This remained true for all passages (1 through 6) and

Table 2 Visceral metastasis production by human colorectal carcinoma cells growing s.c. in nude mice

<table>
<thead>
<tr>
<th>Tumor line</th>
<th>Tumor doubling time (day ± SD)</th>
<th>Day of autopsy*</th>
<th>Tumor wt (g ± SD)*</th>
<th>No. of mice with macroscopic metastases/total</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCC-P4733</td>
<td>7.3 ± 1.2</td>
<td>64 (46–90)</td>
<td>1.48 ± 0.5</td>
<td>0/7</td>
</tr>
<tr>
<td>HCC-P10055</td>
<td>7.1 ± 1.5</td>
<td>77 (43–77)</td>
<td>3.6 ± 1.6</td>
<td>0/6</td>
</tr>
<tr>
<td>HCC-P4491</td>
<td>8.2 ± 1.6</td>
<td>58 (55–63)</td>
<td>1.16 ± 0.5</td>
<td>0/6</td>
</tr>
<tr>
<td>HCC-P8589</td>
<td>5.1 ± 1.3</td>
<td>55 (55–63)</td>
<td>3.38 ± 1.8</td>
<td>1/0</td>
</tr>
<tr>
<td>HCC-M14328</td>
<td>3.5 ± 0.6</td>
<td>30 (30–57)</td>
<td>7.74 ± 5.6</td>
<td>0/6</td>
</tr>
<tr>
<td>HCC-M2193</td>
<td>4.2 ± 1.4</td>
<td>82 (74–83)</td>
<td>4.6 ± 3.7</td>
<td>0/6</td>
</tr>
<tr>
<td>HCC-M1544</td>
<td>4.7 ± 0.5</td>
<td>49 (49–51)</td>
<td>2.2 ± 0.6</td>
<td>0/5</td>
</tr>
<tr>
<td>HCC-M1410</td>
<td>4.7 ± 1.1</td>
<td>50 (47–72)</td>
<td>4.1 ± 3.9</td>
<td>0/10</td>
</tr>
</tbody>
</table>

* Range in parentheses.

† At time of autopsy.

‡ Nine visible metastases 1 to 3 mm in diameter were obtained from the lung of one mouse.
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for all sites of growth. All of the original tumors were histologically grade 2 or 3. One (HCC-M14328) produced large amounts of mucin, a feature that was also present in the mouse xenografts. Neither the xenografts nor the metastases displayed a significant amount of associated fibrosis or inflammatory cell infiltration (Fig. 1).

Lack of Metastasis Formation from s.c. Growing Tumors. In the course of 16 months of this study all mice with tumors growing s.c. were autopsied. The results shown in Table 2 were representative of a large number of similar studies. Grossly visible visceral metastases were produced by only one tumor (HCC-P8589), and this only in one mouse (Table 2). Moreover, histological examination of relevant organs for metastasis of these tumors did not display evidence of tumor growth.

In various experimental rodent tumors, the excision of the primary tumor has been associated with acceleration of metastasis. To examine this possibility in the nude mouse model, cells from the 8 tumor lines were injected into the hind thigh of mice (8 mice per group), and when the tumors reached 1.5–2.0 cm in diameter the leg was amputated at the midfemur. The mice were allowed to survive for up to 6 months after initial implantation of tumor cells. In general, resection of the colorectal carcinomas growing in a “local” site did not increase the production of metastases. However, in two mice, injected with tumors HCC-P4733 and HCC-M2193, macroscopic lung metastases were found. These mice survived 150 and 122 days, respectively, and in both a large tumor recurrence at the site of the initial surgical excision was found.

It should be noted that in the experiments described above, metastases occurred only in one mouse per group and these results were not reproducible in different experiments or passages with the same tumor line. Moreover, increases in metastatic capacity were not observed with continuous passages in nude mice (data not shown).

Experimental Pulmonary Metastasis. The capacity of cells from the 8 colorectal carcinoma lines to produce experimental lung metastases after i.v. injection is shown in Table 3. The experimental metastatic formation among the lines was heterogeneous. For the primary HCC this ranged from a median lung colony of 0 (HCC-P4491) to a median of 48 (HCC-P8589). Cells derived from liver metastases or from the lymph node metastasis produced few lung colonies in nude mice with the exception of line HCC-M1410, which was very metastatic (Table 3).

Production of Liver Metastases Subsequent to i.s. Injection. The capacity of cells from the 8 different HCC lines to produce tumor growth in the liver of nude mice was examined subsequent to injection of cells into the spleen (Fig. 2). The data

Table 3 Production of experimental lung metastases by human colorectal carcinoma cells injected i.v. into nude mice

<table>
<thead>
<tr>
<th>Tumor line</th>
<th>Time of autopsy (days)</th>
<th>No. of mice with lung colonies</th>
<th>Median lung colonies/mouse (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCC-P4733</td>
<td>50</td>
<td>9/9</td>
<td>3 (1–26)</td>
</tr>
<tr>
<td>HCC-P10055</td>
<td>70</td>
<td>6/6</td>
<td>15 (5–29)</td>
</tr>
<tr>
<td>HCC-P4491</td>
<td>90</td>
<td>3/10</td>
<td>0 (0–8)</td>
</tr>
<tr>
<td>HCC-P8589</td>
<td>70</td>
<td>8/8</td>
<td>48 (7–150)</td>
</tr>
<tr>
<td>HCC-M14328</td>
<td>90</td>
<td>7/10</td>
<td>1 (0–12)</td>
</tr>
<tr>
<td>HCC-M2193</td>
<td>90</td>
<td>0/6</td>
<td>0</td>
</tr>
<tr>
<td>HCC-M1544</td>
<td>90</td>
<td>6/10</td>
<td>2 (0–21)</td>
</tr>
<tr>
<td>HCC-M1410</td>
<td>30</td>
<td>8/9</td>
<td>51 (0–161)</td>
</tr>
</tbody>
</table>

Fig. 1. Photomicrographs of HCC-P10055 tumor. Original tumor in colon (a), tumor growing in the spleen of athymic mouse (b), secondary growth in the liver of nude mouse given i.s. injection (c), and tumor growing within the wall of a blood vessel in the liver of a nude mouse (d).
summarized in Table 4 demonstrate that by this criterion the malignant potential of cells derived from hepatic metastases in patients with colorectal carcinoma could be distinguished from that of cells derived from primary colorectal carcinomas.

All tumor cell lines produced growths in the spleens of injected nude mice (30 of 34 mice for primary HCC and 27 of 27 mice given injections of cells from HCC metastases). Autopsies of nude mice began at 30–40 days after i.s. injection of

Fig. 2. HCC tumors growing in the spleens and livers of athymic nude mice given injections of HCC-P4733 (a), HCC-P10055 (b), HCC-P4491 (c), HCC-P8589 (d), HCC-M14328 (e), HCC-M2193 (f), HCC-M1544 (g), and HCC-M1410 (h). Note the presence of grossly visible liver metastases (arrows). For time of autopsy and relative size of spleen tumors, see Table 4.
cells. At this time, no liver lesions were observed in mice given injections of cells from colorectal carcinoma cell lines established from primary neoplasms. In contrast, mice given injections of HCC-M1544 or HCC-M1410 cells became moribund at 40 or 30 days, respectively, after i.s. injection. At this time the mice were autopsied, and their livers were completely replaced by tumor deposits (Fig. 2). Few or no liver metastases were observed after the i.s. injection of the HCC-M14328 and HCC-M2193 cell lines (Table 4; Fig. 2).

The degree of tumor growth in the livers of nude mice did not depend on the size of tumors growing in the spleens (Table 4; Fig. 2). Cells from lines established from primary HCC cells produced spleen tumors, but at 40 days after i.s. injection, no liver growths were found. In mice given injections of cells isolated from primary colorectal carcinoma, autopsies at 90 days after i.s. injection revealed the presence of liver metastases, albeit fewer than observed in mice given injections of cells from metastatic lines at days 30 or 40 after i.s. injection (Table 4). Cells from line HCC-P8589 produced liver metastases in all the injected mice, whereas the other 3 primary derived lines produced metastases in about 50% of the mice at the time of autopsy (90 days after i.s. injection). Cells from HCC-P10055 produced micrometastases in the liver that were detected only after microscopic histological examinations (Fig. 1).

DISCUSSION

The implantation of metastatic human colorectal carcinoma cells into the spleen of athymic nude mice was followed by growth of the tumors in the liver and the death of the recipient animal. In contrast, the injection of the same population of cells into the subcutis or musculature of nude mice produced only local tumors without systemic metastases. Indeed, we studied over 200 mice with HCC growing s.c. and in only one mouse did we find macroscopic evidence of visceral (lung) metastases at time of autopsy. Growth of HCC in the hind thigh (i.m.) with or without amputation of the local tumor also did not produce frequent metastases. Only in a very few mice did we find lung metastases at 6 months after tumor implantation, resection, and local tumor recurrence. Whether the metastases that we observed were due to a growth of tumor cells already present in the lung at the time of the primary tumor excision, to a successive dissemination of cells from tumor recurrence, or to the shedding of tumor cells into blood vessels consequent to surgical manipulation is unclear (13, 16, 26, 27).

The rarity of metastasis production by human tumors implanted s.c. or i.m. into nude mice is well documented (6, 13, 15). The relatively brief life span of nude mice compared with human beings may explain why metastasis of human tumor cells occurs so infrequently. However, many other factors may influence this process in a more profound manner. (a) The majority of human neoplasms xenografted into nude mice have been implanted s.c., an anatomical site that bears little relevance to the organ of origin for the neoplasm, e.g., colorectal carcinomas. In this regard, it is interesting to note that the implantation of a human colon carcinoma line into the gut of nude mice was followed by extensive invasion and lymph node metastases, but this phenomenon was not found for gut-implanted melanoma (22). (b) The metastatic capacity of human tumor cells implanted s.c. in nude mice has been correlated with invasion of the body wall (11, 12, 15). Thus, the lack of invasion and metastasis by human tumor cells has often been associated with the presence of a fibrous capsule (of mouse origin) surrounding the locally growing human tumor (28).

We questioned the validity of implanting human colorectal carcinomas into the subcutis or muscularis of nude mice. Implantation at these sites may be relatively easy to accomplish, but its relevance to the biology of colorectal carcinoma is open to severe questioning. The i.s. injection of some human tumor cell lines (melanoma, colon carcinoma, prostatic carcinoma) (23) and mouse Lewis lung carcinoma (29) has been shown to produce extensive liver and lung metastasis. The liver metastasis could not have been due to simple drainage of cells from the spleen. We base this conclusion on the data showing that human renal cell carcinomas implanted i.s. can produce tumors in the spleens of nude mice without liver metastases. In fact, these tumors produced lung metastases in the injected mice (23). We now confirm these earlier results obtained with long established cell lines and extend the findings to tumor cells recently isolated from HCC.

Human tumors xenografted into the nude mice have a long latency at the first transplant generation, but with further passages they grow more rapidly, and the growth characteristics tend to stabilize (30, 31). We determined the metastatic potential of primary and metastatic HCC subsequent to 3–6 passages in nude mice. Although selection of aggressive cells could indeed have occurred by the time of s.c. passage of the tumors, we observed differences in hepatic metastatic potential (Table 4), and this agreed with a previous report that serial passages of human tumors in nude mice do not induce a significant increase in metastatic capacity (11, 13, 15, 30). Indeed, despite a generally higher percentage of tumor takes at later passages, the tumor lines we examined maintained their metastatic phenotype at different passage generations (data not shown). Furthermore, although the most malignant lines were also the fastest growing (HCC-M1544, HCC-M1410), the capacity to form tumor deposits in the liver or in the lung did not necessarily correlate with s.c. growth or growth in the spleen (HCC-M2193, HCC-14328) (Table 2; Fig. 2).

The implantation of HCC cells into the spleen of athymic nude mice allowed us to correlate the malignant behavior of

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### Table 4 Liver metastases produced by human colorectal carcinoma cells injected into the spleens of nude mice

<table>
<thead>
<tr>
<th>Tumor line</th>
<th>Time of autopsy (day)</th>
<th>No. of mice with tumors in the spleen</th>
<th>No. of mice with liver deposits</th>
<th>Liver tumor burden</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCC-P4733</td>
<td>90</td>
<td>8/9 (L)</td>
<td>4/9</td>
<td>0, 0, 0, 0, 0, 0</td>
</tr>
<tr>
<td>HCC-P10055</td>
<td>90</td>
<td>9/10 (M)</td>
<td>10/10</td>
<td>I, I, I, I, I, I</td>
</tr>
<tr>
<td>HCC-P4491</td>
<td>90</td>
<td>8/10 (S)</td>
<td>7/10</td>
<td>0, 0, 0, 0, 0, 0</td>
</tr>
<tr>
<td>HCC-P8589</td>
<td>90</td>
<td>5/5 (L)</td>
<td>5/5</td>
<td>II, III, III, III</td>
</tr>
<tr>
<td>HCC-M14328</td>
<td>90</td>
<td>6/6 (L)</td>
<td>1/6</td>
<td>0, 0, 0, 0, 0, 0</td>
</tr>
<tr>
<td>HCC-M2193</td>
<td>90</td>
<td>6/6 (L)</td>
<td>2/6</td>
<td>0, 0, 0, 0, 0, L</td>
</tr>
<tr>
<td>HCC-M1544</td>
<td>90</td>
<td>6/6 (S)</td>
<td>6/6</td>
<td>IV, IV, IV, IV, IV</td>
</tr>
<tr>
<td>HCC-M1410</td>
<td>30</td>
<td>9/9 (M)</td>
<td>9/9</td>
<td>IV, IV, IV, IV, IV</td>
</tr>
</tbody>
</table>

* Tumor burden in the liver was graded as described in "Materials and Methods."
tumors in the patients and in the nude mice. Specifically, by days 30–40 after injection of HCC-M1410 or HCC-M1544 cells, the nude mice became moribund. Autopsy revealed that their liver was totally replaced by neoplastic growth, which was ascertained to be HCC by histological, isozyme, and karyotypic analysis. No gross liver metastases were found in nude mice killed 40 days after the i.s. injection of cells from primary HCC. Thus, if the “metastatic potential assay” was terminated after 30–40 days, quantitative differences between metastatic and nonmetastatic HCC cells were clearly observed. If nude mice given i.s. injections of primary HCC cells were allowed to survive for 90 days, liver metastases were observed, albeit at a much reduced incidence. Whether the hepatic nodules in the nude mice produced by primary HCC cells represent a selected subpopulation is not clear at present, and these studies are under way.

The metastatic process is the end result of a complex sequence of events. The i.v. or i.s. injection of tumor cells bypasses the restriction of local growth but still requires tumor cells to survive in the blood stream, invade, and above all grow in distant organs. Correlation or near correlation was observed in the capacity of cells to grow in the liver and colonize the lung after injecting HCC cells into the spleen or i.v. (Tables 3 and 4). For example, cells from HCC-M1410 and HCC-P8589 were highly metastatic following both routes of injection, suggesting that these tumor cells were able to arrest and grow in distant visceral organs. On the other hand, cells from HCC-M14328 (a lymph node metastasis) and HCC-M2193 (liver metastasis) produced large s.c. and spleen tumors but grew poorly in the liver or in the lung. In this regard it is interesting to observe that in two separate experiments, the HCC-M14328 cells injected into the spleens of nude mice produced metastases in the ovaries of the mice, suggesting a more complex organ arrest pattern intrinsic to these tumor cells (data not shown). Unfortunately, the limited number of female nude mice in our facility prevented an extensive comparison with the other tumor lines. The findings that the HCC-M1544 produced extensive tumors in the liver, but poor lung growth, support the interpretation that the environment at the site of arrest, together with intrinsic characteristics of tumor cells, influences metastatic development (32).

In conclusion, our studies demonstrate that under specific transplantation procedures, HCC cells injected into nude mice exhibit different metastatic capacities. In particular, the injection of HCC cells into the spleens of nude mice may represent an advantageous route of injection for determining the malignant potential of human colorectal carcinomas.

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20. Kyrizias, A. P., DiPersio, L., Michael, G. L., Peace, A. M., and Stinnett, J. A. The metastatic process is the end result of a complex sequence of events. The i.v. or i.s. injection of tumor cells bypasses the restriction of local growth but still requires tumor cells to survive in the blood stream, invade, and above all grow in distant organs. Correlation or near correlation was observed in the capacity of cells to grow in the liver and colonize the lung after injecting HCC cells into the spleen or i.v. (Tables 3 and 4). For example, cells from HCC-M1410 and HCC-P8589 were highly metastatic following both routes of injection, suggesting that these tumor cells were able to arrest and grow in distant visceral organs. On the other hand, cells from HCC-M14328 (a lymph node metastasis) and HCC-M2193 (liver metastasis) produced large s.c. and spleen tumors but grew poorly in the liver or in the lung. In this regard it is interesting to observe that in two separate experiments, the HCC-M14328 cells injected into the spleens of nude mice produced metastases in the ovaries of the mice, suggesting a more complex organ arrest pattern intrinsic to these tumor cells (data not shown). Unfortunately, the limited number of female nude mice in our facility prevented an extensive comparison with the other tumor lines. The findings that the HCC-M1544 produced extensive tumors in the liver, but poor lung growth, support the interpretation that the environment at the site of arrest, together with intrinsic characteristics of tumor cells, influences metastatic development (32).

In conclusion, our studies demonstrate that under specific transplantation procedures, HCC cells injected into nude mice exhibit different metastatic capacities. In particular, the injection of HCC cells into the spleens of nude mice may represent an advantageous route of injection for determining the malignant potential of human colorectal carcinomas.
Metastatic Behavior of Tumor Cells Isolated from Primary and Metastatic Human Colorectal Carcinomas Implanted into Different Sites in Nude Mice

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