Growth and Metastasis of Tumor Cells Isolated from a Human Renal Cell Carcinoma Implanted into Different Organs of Nude Mice

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ABSTRACT

The purpose of this study was to determine whether the methods for isolating tumor cells from a human renal cell carcinoma (HRCC) influence the biological behavior of the cancer cells. Renal cell carcinoma obtained from a surgical specimen was dissociated by enzymatic treatment and cells were plated into culture dishes or injected s.c. into the kidney of BALB/c nude mice. The resultant kidney tumor produced liver metastasis and ascites. All tumors growing in nude mice (s.c., kidney, liver, ascites) were also established in culture. The human origin of all five lines was ascertained by karyotypic and isoenzyme analyses. Cells from all lines were injected, i.p., i.v., intrasplenic, and beneath the renal capsule of nude mice. All the lines were tumorigenic after s.c. or renal subcapsule injection, although the rate of tumor growth varied among the five lines. The metastatic behavior of the HRCC cells was influenced by both the nature of the tumor cells and the route of injection into nude mice. In general, cells derived from the liver metastasis produced more metastases in nude mice than other lines. The lines established in culture from the primary HRCC and the ascites were poorly metastatic. Even with highly metastatic cells, i.v. injection did not yield significant metastasis, but the injection of cells into the renal subcapsule resulted in extensive metastasis to the lungs and in all peritoneal organs.

These results indicate that nude mice can be used for the isolation of populations of HRCC cells with different growth and metastatic potential and that, of the organ sites tested, the renal subcapsule is the most advantageous site for implantation of HRCC cells.

INTRODUCTION

The concept that neoplasms are heterogeneous and contain subpopulations of cells having different biological behavior patterns, including metastatic potential, has gained wide acceptance (1–6). The outcome of cancer metastasis in rodents and in humans has been shown to depend on the interaction of metastatic tumor cells with host factors, including organ environment (3–7). Most of the data on metastatic heterogeneity and biological behavior of neoplasms have been derived from studies in rodent systems, and little data are available on the in vivo metastatic process by freshly isolated human cancers. To a great extent this has been due to the lack of suitable models for the isolation of appropriate human cancer cells (i.e., nonmetastatic or metastatic) and the lack of a suitable laboratory model for the in vivo studies of human cancer metastasis. Since Ryagaard and Povlsen (8) reported that xenogeneic human tumors could grow inathymic T-cell-deficient nude mice, the availability of nude mice for studies of heterotransplantation of cells or tissues has proved valuable in studying many aspects of human tumor biology (8–19). Although human tumors grown in nude mice maintained their original morphological and biochemical characteristics, even highly malignant neoplasms rarely produced metastasis in the nude mouse recipient (9, 11, 13, 18).

Because metastasis is influenced by both tumor cell properties and host factors (1, 4–6), it is not surprising that even in nude mice the outcome of metastasis depends on the nature of the tumor cells, on experimental conditions such as route of administration or organ for implantation (20–29), and on the source of the mice used (15, 30, 31).

The site of tumor growth can influence the production of metastasis (4). Recent reports from our laboratory have shown that the i.s. injection of human tumor cells derived from several well-established cell lines (colon carcinoma, prostatic carcinoma) (19) or tumor cells freshly isolated from human colorectal carcinomas (32) allowed for the expression of metastatic potential, whereas implantation of the same cells into the subcutis did not. One notable exception was an established HRCC line in which growth of cells in the spleen was not associated with production of metastases in the liver or lung (19).

In the present study we determined whether freshly isolated cells from a HRCC exhibited biological heterogeneity in vitro and, after implantation in athymic nude mice, in vivo. Because the 5 cell lines of the HRCC were derived from 5 different isolation conditions (culture, s.c. tumor, renal tumor, liver metastasis from the renal tumor, ascites from the renal tumor), we asked whether the cell lines exhibited biological heterogeneity, including metastatic potential. Finally, we also examined the issue of appropriate modeling for metastasis of HRCC. The availability of these 5 lines allowed us to conclude that they consisted of different subpopulations of cells with diverse growth and metastatic potential and also to demonstrate that the implantation of HRCC cells into the kidney of nude mice provided the most advantageous method of examining their malignant potential.

MATERIALS AND METHODS

Animals. Male athymic BALB/c nude mice were obtained from the Animal Production Area of the NCI-Frederick Cancer Research Facility, Mice were used when 8 weeks old and were maintained in a laminar flow cabinet under specific-pathogen-free conditions.

Tissue Culture Conditions. All tumor cell lines were maintained on plastic as monolayer cultures in Eagle’s minimum essential medium (M. A. Bioproducts, Walkersville, MD) supplemented with 10% fetal bovine serum, sodium pyruvate, nonessential amino acids, L-glutamine, and a 2-fold vitamin solution (GIBCO, Grand Island, NY). All the cell lines were examined for and were found to be free of Mycoplasma, reovirus type 3, pneumonia virus of mice, mouse adenovirus, murine hepatitis virus, lymphocytic choriomeningitis virus, ectromelia virus, and lactate dehydrogenase virus (Microbiological Associates, Bethesda, MD).

Establishment of Tumor Cell Lines. The origin of the 5 lines of HRCC is schematically presented in Fig. 1. The tumor tissue was obtained from a primary renal tumor subsequent to a radical nephrectomy in a 43-year-old male. The tumor was diagnosed as a renal cell carcinoma with extensive invasion of perinephric fat (Fig. 2a). Tumor...
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Fig. 1. Methods and procedures used to isolate 5 different lines of HRCC from a surgical specimen.

The cells from these 5 lines grew on plastic as a monolayer culture and showed quite similar morphology (Fig. 2, b-f). The human origin of all 5 lines was ascertained by detailed karyotypic analysis and showed quite similar morphology (Fig. 2, b-f). The human origin of all 5 lines was ascertained by detailed karyotypic analysis and showed quite similar morphology (Fig. 2, b-f). The human origin of all 5 lines was ascertained by detailed karyotypic analysis and showed quite similar morphology (Fig. 2, b-f).

In Vitro Growth Rate Determinations. Cells from ninth passage generation were harvested from subconfluent cultures by overlaying monolayers with a solution of 0.25% trypsin and 0.02% EDTA. After 1 min the flasks were tapped sharply to dislodge the cells, which were then washed in medium with serum and resuspended in HBSS for injection. Only single cell suspensions with a viability greater than 90% viability were used in the in vitro experiments.

Tumor Cell Injections s.c. Mice were given injections, at the central s.c. site, of 1 x 10^6 viable tumor cells suspended in 0.2 ml of HBSS. Tumor growth s.c. was measured weekly, and tumor volume was calculated as

\[ \text{Tumor volume} = \frac{\text{length} \times (\text{width}^2)}{2} \]

Tumor weight values were plotted against days postinjection. Tumor doubling time was thus calculated. When s.c. tumors reached 2 cm in diameter, the mice were killed. Visceral organs were removed and rinsed in water, and the lungs were inflated and fixed in Bouin's solution. After fixation for 24 h, lungs were examined under a dissecting microscope, and the number of peripheral lung tumor nodules larger than 0.1 mm in diameter were determined. The neoplastic nature of these lung nodules was confirmed by histological examination. All the s.c. tumors and visceral organs with suspected metastasis were fixed in 10% buffered formalin solution and examined histologically.

Tumor Cell Injections i.v. To produce pulmonary tumor colonies (experimental metastases), mice were given i.v. injections of 1 x 10^6 viable tumor cells in 0.2 ml HBSS through the lateral tail vein. Eight weeks later the mice were killed, the lungs fixed in Bouin's solution, and the number of peripheral tumor nodules was determined.

Tumor Cell Injections i.p. Mice were given i.p. injections of 1 x 10^6 viable tumor cells in 0.2 ml HBSS. Eight weeks after the injection of tumor cells, mice were killed and autopsied, and visceral organs were collected and examined for the presence of tumor growth by gross and histological criteria.

Tumor Cell Injections i.s. After the mice were anesthetized with methoxyflurane, we injected into the spleen 1 x 10^6 viable tumor cells suspended in 0.05 ml of HBSS according to the methods described in detail previously (19). Eight weeks after the injection of tumor cells, the mice were killed and autopsied. All visceral organs were examined by gross and histological procedures to verify tumor growth.

Tumor Cell Injection into the RSC. Mice were anesthetized with methoxyflurane and placed in the left lateral decubitus position. A vertical incision was made in the right flank through the skin and peritoneum, exposing the lateral aspect of the kidney. The kidney was lifted gently and stabilized. A 27-gauge needle was inserted into the renal parenchyma from the lower pole of the kidney and advanced until its tip reached just below the renal capsule. At this time the mice were given injections of 1 x 10^6 viable tumor cells in 0.05 ml HBSS. A visible bulia formation between renal parenchyma and capsule and the lack of significant bleeding or extrarenal leaking of the injected tumor cell suspension were the criteria for a successful injection. After injection, the kidney was returned to the abdominal cavity, and the wound was closed in one layer with wound clips. When the mice became moribund they were autopsied. The injected kidney was removed and fixed in 10% buffered formalin solution after measuring the weight. All visceral organs were fixed in formalin or Bouin's solution. Determinations of metastasis were carried out after gross and histological examination.

Statistical Analysis. The results of all in vivo studies were analyzed by the Mann-Whitney U test (2-tailed).

RESULTS

Growth Rate in Vitro and Tumor Growth and Metastasis after s.c. Injection. The growth rates of the cells from the five lines in vitro and in the subcutis of nude mice are shown in Table 1. The tumor doubling time in vitro did not differ significantly among the lines, with the exception of the SN12C line initially adapted to culture, which exhibited the shortest doubling time. In contrast, the median tumor doubling time of each line at a s.c. site of nude mice differed, ranging from 5.2 to 11.9 days. All lines were tumorigenic, but SN12L1 cells grew most rapidly. The growth rate of SN12K1 at the s.c. site was the slowest among the lines. A significant difference was observed in the growth rate of SN12L1 and SN12C (P < 0.005), SN12K1 (P < 0.005), or SN12A1 (P < 0.005). No direct relationship was found between the growth rates of tumor cells in vitro and at a...
s.c. site. Irrespective of the tumor cell lines, a large part of the growing tumor in the subcutis was necrotic. The tumors contained a large quantity of necrotic fluid, and occasionally their surface was ulcerated. This was especially noticeable in the tumors produced by cells from the SN12S1 line.

The metastatic behavior of the five lines after s.c. injection is also shown in Table 1. Spontaneous metastasis to lungs of mice from s.c. tumors was found in most of the animals. The number of lung tumor colonies, however, differed among the various groups. The highest number of lung metastases was produced by cells of SN12S1 (skin isolated tumor) and the lowest number of pulmonary metastases was produced by cells of SN12A1 (ascites-isolated tumor). Extrapulmonary metastases were found in only one mouse given an injection of SN12K1 and one mouse given an injection of SN12L1 cells, in which metastasis to the axillary lymph node was found.

Experimental Pulmonary Metastasis. The production of experimental lung metastases by HRCC cells injected i.v. into
Table 1 Growth rates in vitro and at a s.c. site and production of spontaneous metastasis

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Doubling time in vitro (h)</th>
<th>s.c. growth</th>
<th>Pulmonary metastasis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Incidence</td>
<td>Median tumor doubling time in days (range)</td>
<td>Incidence</td>
</tr>
<tr>
<td>SN12C</td>
<td>25.9</td>
<td>5/6</td>
<td>9.4 (8.1–10.8)</td>
</tr>
<tr>
<td>SN12S1</td>
<td>29.5</td>
<td>5/6</td>
<td>7.6 (4.9–8.4)</td>
</tr>
<tr>
<td>SN12K1</td>
<td>30.0</td>
<td>5/6</td>
<td>11.9 (7.6–14.7)</td>
</tr>
<tr>
<td>SN12L1</td>
<td>30.5</td>
<td>7/7</td>
<td>5.2 (4.6–7.0)</td>
</tr>
<tr>
<td>SN12A1</td>
<td>30.0</td>
<td>5/6</td>
<td>9.0 (7.8–10.5)</td>
</tr>
</tbody>
</table>

Table 2 Production of experimental pulmonary metastasis by HRCC cells injected i.v. into nude mice

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Incidence</th>
<th>Median nodules/mouse (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SN12C</td>
<td>1/6</td>
<td>0 (0–2)</td>
</tr>
<tr>
<td>SN12S1</td>
<td>6/6</td>
<td>1 (1–2)</td>
</tr>
<tr>
<td>SN12K1</td>
<td>4/6</td>
<td>2 (0–13)</td>
</tr>
<tr>
<td>SN12L1</td>
<td>4/5</td>
<td>4 (0–12)</td>
</tr>
<tr>
<td>SN12A1</td>
<td>0/6</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

nude mice varied among the lines. The data shown in Table 2 demonstrate that cells of line SN12L1 (line isolated from a liver metastasis) produced more lung metastases than did cells of line SN12C (line isolated in culture) or the SN12A1 line (ascites-isolated line). The difference, however, was not significant because the median number of lung tumor colonies was quite small regardless of the line injected.

Tumor Growth and Metastasis after i.p. Injection. Tumor growth and production of local and systemic metastasis after the i.p. injection of tumor cells are shown in Table 3. Here again, the behavior of the 5 lines differed markedly. Cells from the SN12A1 line originally isolated from an ascitic tumor produced the highest incidence of ascites (5 of 6 mice). Cells from SN12C (tissue culture) or SN12L1 (liver metastasis) produced ascites in only 2 of 6 animals. In the majority of mice with ascites, tumor growth on the surface of peritoneal organs was very evident (Fig. 3a). Cells from the SN12L1 line were highly invasive (despite the low incidence of ascites). With the exception of line SN12K1 (kidney isolated line), the incidence of pulmonary metastasis was generally low, and even in mice in whom the metastases appeared the number of lung tumor nodules was small.

Growth and Metastasis after i.s. Injection. The intrasplenic growth of tumor cells and production of systemic metastasis are shown in Table 4. The ability to grow in the spleen and produce systemic metastasis varied among the 5 cell lines. After i.s. injection most of the mice given injections of cells from lines SN12C, SN12S1, and SN12K1 developed grossly evident tumors in the spleen, the pancreas, or both. In contrast, the i.s. injection of cells from lines SN12L1 produced more tumors in the pancreas than in the spleen. Cells from the SN12A1 line were relatively nontumorigenic after i.s. injection. This was in sharp contrast to their tumorigenic potential after i.p. injection (Table 3).

The incidence of systemic visceral metastasis (lung, liver, kidney) was low for all the lines; however, peritoneal lymph nodes, including omental and mesenteric nodes, were extensively involved in the mice given injections of the cells from SN12K1 and SN12L1 lines.

Tumor Growth and Metastasis after Injection into the RSC. The incidence of tumor growth and metastasis after injection of HRCC cells into the RSC is shown in Table 5. We placed into the RSC of mice injections of 1 × 10^6 viable cells and killed the mice when they were moribund. The injection of HRCC cells into the kidney environment produced the highest incidence of tumorigenicity regardless of the cell line (Fig. 3b), suggesting that the kidney is a better environment for expression of tumorigenicity of HRCC cells than the subcutis, veins, spleen, or peritoneum. The growth rate of the HRCC cells in the RSC varied among the lines. The fastest growing tumors were produced by cells from SN12L1 line (liver metastasis). Animals given injections of these cells became moribund by 5 weeks after RSC injection. The slowest growing tumors were produced by cells of the SN12C (tissue culture) or SN12A1 (ascites derived) lines. Animals became moribund by 8 weeks after RSC injection. By the time of autopsy, however, all renal tumors attained the same size (2.6–3.0 g). No simple correlation was found between the growth rate of the cells in vitro (Table 1) and s.c. (Table 1), i.v. (Table 2), i.p. (Table 3), i.s. (Table 4), or RSC (Table 5) injections. For example, SN12L1 cells, with the slowest growth rate in vitro, grew most rapidly in the kidney, and SN12C cells, with the most rapid growth rate in vitro, grew most slowly in the kidney.

The production of pulmonary metastasis from kidney tumors varied among the lines. Once again, a high incidence of lung metastasis was observed with cells of line SN12L1 (derived from a liver metastasis). In sharp contrast, cells of the SN12A1 line (ascites derived) produced few lung metastases (Fig. 3c). These differences in production of lung metastases were highly significant when SN12L1 and SN12C or SN12A1 cells were compared (P < 0.005). In addition to causing lung metastasis, cells from the SN12L1 line were highly invasive and produced a high incidence of metastasis in the omental and mesenteric lymph nodes, pancreas, diaphragm, and seminal vesicles. Moreover, in 4 of 6 mice with kidney tumors, liver metastases occurred (Fig. 3d). In 5 of 6 mice ascites were found. This high incidence of metastasis was attributed to the malignant nature of the SN12L1 cells, since cells of the other 4 lines failed to produce this metastatic pattern. Moderate metastatic potential was observed with cells from lines SN12S1 and SN12K1. Low metastatic potential was observed with cells of lines SN12C and SN12A1.

DISCUSSION

We succeeded in producing 5 HRCC cell lines with continuous growth in vitro. The large HRCC specimen yielded a

Table 3 Growth and metastasis of HRCC lines injected i.p. into nude mice

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Incidence of tumor ascites</th>
<th>Incidence</th>
<th>Median nodules/mouse (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SN12C</td>
<td>2/6</td>
<td>1/6</td>
<td>0 (0–8)</td>
</tr>
<tr>
<td>SN12S1</td>
<td>4/6</td>
<td>2/6</td>
<td>0 (0–12)</td>
</tr>
<tr>
<td>SN12K1</td>
<td>3/5</td>
<td>4/5</td>
<td>2 (0–21)</td>
</tr>
<tr>
<td>SN12L1</td>
<td>2/6</td>
<td>1/6</td>
<td>0 (0–2)</td>
</tr>
<tr>
<td>SN12A1</td>
<td>5/6</td>
<td>2/6</td>
<td>0 (0–3)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tumor growth in</th>
<th>Liver</th>
<th>Kidney</th>
<th>Pancreas</th>
<th>Seminal vesicles</th>
<th>Diaphragm and lymph nodes</th>
</tr>
</thead>
<tbody>
<tr>
<td>SN12C</td>
<td>0/6</td>
<td>0/6</td>
<td>2/6</td>
<td>1/6</td>
<td>3/6</td>
</tr>
<tr>
<td>SN12S1</td>
<td>2/6</td>
<td>0/6</td>
<td>5/6</td>
<td>5/6</td>
<td>5/6</td>
</tr>
<tr>
<td>SN12K1</td>
<td>1/5</td>
<td>1/5</td>
<td>3/5</td>
<td>4/5</td>
<td>5/5</td>
</tr>
<tr>
<td>SN12L1</td>
<td>1/6</td>
<td>2/6</td>
<td>6/6</td>
<td>4/6</td>
<td>6/6</td>
</tr>
<tr>
<td>SN12A1</td>
<td>0/6</td>
<td>0/6</td>
<td>4/6</td>
<td>4/6</td>
<td>5/6</td>
</tr>
</tbody>
</table>
sufficient number of cells to allow simultaneous establishment of in vitro culture (SN12C) and the injection of the HRCC cells into a s.c. site or the kidney of nude mice. The cell lines SN12S1 and SN12K1 were derived from the HRCC tumors growing in the skin and kidney of nude mice, respectively. Metastasis to the liver and ascites were found in the nude mice with HRCC growing in the kidney. The continuous in vitro lines SN12L1 and SN12A1 were derived from the liver nodule and the ascites, respectively. The human origin of all the 5 HRCC lines was ascertained by karyotypic analysis and by isoenzyme analysis. Because the 5 lines were derived from a single HRCC the demonstration of biological diversity among the lines indicate that the tumor consisted of different subpopulations of cells (i.e., the original HRCC was biologically heterogeneous).

The heterogeneous nature of many animal and human neoplasms is well documented (1–6, 15–17, 19, 28, 33). The present study not only supports this conclusion but also adds new and important information. First, the biological behavior of HRCC cells isolated by direct culture technique (SN12C) and the cells isolated from HRCC tumors and grown first in the nude mice and then established in culture differed. This difference raises a question about which method should be used routinely if human tumor cell lines are to be isolated either for studies of biology or for predictive sensitivity for therapeutic agents.

Second, cell lines derived from HRCC tumors produced by the original HRCC cells injected into the skin (SN12S1) or into the kidney of nude mice (SN12K1) differed in their biological properties. Moreover, cell lines derived from a liver metastasis (SN12L1) and ascites (SN12A1) produced by the original HRCC cells growing in the kidney of a nude mouse also exhibited unique biological properties. This finding raises a second question about which organ site of nude mice should be
used for the transplantation of heterogeneous human tumors. In other words, the routine transplantation of human tumors such as colorectal carcinoma, lung carcinoma, HRCC, and others into the s.c. space of nude mice is relatively easy, but does it necessarily yield cell lines that resemble the original human tumor? If a human tumor is biologically heterogeneous, some of its cells may possess a growth advantage in one anatomic site or another. The growth of HRCC in the skin or the kidney of nude mice could, therefore, favor the proliferation of different cells.

The growth rate of cells from the 5 lines varied under both in vitro and in vivo (s.c. or kidney implantation) conditions. No direct correlation among growth in vitro, in the s.c. site, or in the kidney was found. The growth rate of tumor cells in vitro can be regulated by manipulation of the environment (i.e., by serum, media, temperature) (34). Similarly, different organ environments influence the growth of some but not all tumor cells (4, 7, 21, 22, 24, 27-29, 33). Indeed, this specific interaction of tumor cells with host environment was the basis for the original "seed and soil" hypothesis by Paget (3).

The growth of the HRCC in the skin, kidney, and liver metastasis and in the ascites form in the nude mice must have selected for cells with different metastatic properties. We base this conclusion on the following. (a) The ranking of cell lines for metastatic propensity was very similar regardless of the site of implantation. Cells of the SN12L1 line were most metastatic followed by cells from SN12S1, SN12K1, SN12C, and SN12A1 lines. SN12L1 cells exhibited invasive and metastatic properties regardless of organ site for their implantation (s.c., i.v., i.p., or RSC). In contrast, cells of the SN12C or SN12A1 lines were poorly metastatic regardless of the site of implantation. (b) The metastatic properties, in nude mice, of the cells were stable despite 8-week assays, and expression of full metastatic pattern (pulmonary and extrapulmonary foci) required that the HRCC cells be implanted into the kidney.

Although metastasis of human neoplasms transplanted into nude mice has been considered to be rare (9, 10, 14, 18), recent studies have indicated that the incidence of metastasis in nude mice can be increased by simple manipulation of the route of tumor cell injection (19, 23, 24). Kyrizias et al. (23) injected cells s.c. or i.p. from human tumor lines of larynx or colon carcinoma into nude mice and showed that only tumors growing i.p. produced metastasis to the lung. Moreover, human tumors implanted in the cranial s.c. site of nude mice grew faster and metastasized more frequently than the same tumors implanted in the posterior aspect of the trunk (24). Kozlowski et al. (19) demonstrated that the i.s. injection, but not i.v. injection, allowed the most dramatic expression of metastatic capacity in several human tumor cell lines with the exception of a HRCC line. Furthermore, most recent studies from our laboratory (32) demonstrated that the expression of metastatic potential of freshly isolated human colorectal carcinomas could be achieved after i.s. injection and not after i.v., s.c., or i.m. injection. Although the implantation of cells into the spleen is most advantageous for human colorectal carcinoma cells (32), it was not for the HRCC cells (Table 4); these data agree with our earlier data using established HRCC cell lines (19).

The full expression of metastatic potential of HRCC cells was obtained after growth in the kidney by some but not by all the HRCC cells. These results reemphasize that both tumor cell properties and host factors determine the outcome of metastasis (5, 6). The s.c. implantation of the HRCC cells into the nude mice produced tumors with a high degree of necrosis. Interestingly, spontaneous lung metastases were produced by cells of the SN12S1 line (originally isolated from a tumor growing s.c.) but not from other lines.

Since the conversion from solid tumor to ascitic form by i.p. passaging of cells could result in an increased metastatic capacity of the tumor cells (35-37), we expected cells of the SN12A1 line to have high metastatic potential. This was not the case, but the SN12A1 line was not derived from a solid tumor artificially converted to ascites tumor but rather from an ascites tumor that spontaneously occurred in a mouse with a tumor growing in the kidney. This line did produce the highest incidence of ascitic tumors after i.p. implantation, suggesting once again that the process for its origin was selective. Nearly 30 years ago, Klein (36) demonstrated that the gradual conversion of certain solid neoplasms into ascites tumors represented the selective overgrowth of a small number of cells that differed from the majority of the original population with regard to their capacity to multiply under the environmental conditions of the peritoneal fluid. Line SN12A1 could well be a selected population from the HRCC tumor having preferential growth in the peritoneal cavity. Growth in the peritoneum, however, and metastatic potential did not correlate in our studies. The most metastatic line, SN12L1, did not produce a high incidence of ascites subsequent to i.p. implantation (Table 3).

In conclusion, nude mice can be used effectively to select HRCC variant lines with different and stable metastatic properties. The expression of metastatic potential of HRCC in nude mice depends on both the intrinsic properties of the HRCC cells and the organ site of their implantation. For renal cell carcinoma, the kidney is the natural organ of implantation which allows full expression of metastatic potential.

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