Prostate Cancer and Other Xenografts from Cells in Peripheral Blood of Patients

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Abstract

Good models for the investigation of human prostate cancer are few. Cells from approximately 9.2–21 ml of peripheral blood from patients with metastatic prostate cancer or metastatic colon cancer were injected s.c. into nude mice. Prostate cancer from 2 of 11 patients and colon cancer from 1 of 3 patients were found to be growing as metastases in the lungs of the nude mice. To our knowledge, this is the first report of the formation of xenografts from carcinoma cells taken directly from the peripheral blood of patients. Expanding circulating cancer cells with this approach may have important translational applications including: (a) development of models of human cancers; and (b) sampling of cancers from specific patients for novel molecular and therapeutic approaches.

Introduction

The treatment of metastatic prostate cancer that is refractory to hormonal manipulation and of many other metastatic cancers is difficult. Although different proportions of metastatic, hormonally refractory prostate cancers respond to different therapeutic approaches, it is very difficult to predict which kinds of therapeutic approaches may benefit particular patients. Many other kinds of metastatic carcinoma present similar problems. One might hope that molecular or pharmacological studies of particular patients’ metastatic tumors might allow the development of therapeutic approaches to benefit particular patients. Characterization or growth in the laboratory of primary tumors might be a useful step for some tumors; however, as reviewed previously in detail (1), for many years it has been known that patients diagnosed with prostate cancer usually have more than one cancer (2), even in the very earliest diagnosed stages of the disease (3). These multiple, widely separated, primary prostatic carcinomas usually show different genetic aberrations (4) and are thought to be independent in origin. It would be difficult to predict in patients which of multiple primary prostatic carcinomas gave rise to metastases. If one wished to characterize the cells from metastases in a patient with a large tumor burden, it might be useful to examine circulating carcinoma cells. We now report the growth of these circulating cells in nude mice. The resultant xenografts might be useful for studies of cancers from particular patients and for the development of prostate cancer xenografts for a variety of research purposes.

Materials and Methods

Blood Cells for Mice. In response to our request for 10 ml of blood from patients with advanced, treatment-refractory metastatic cancer, we received 9.2–21 ml of blood from 14 patients (11 patients with prostate cancer and 3 patients with colon cancer). Blood from patients in this study was obtained while consistently maintaining the anonymity of the patients. Because the cells from one of the patients with metastatic prostate cancer caused more frequent swelling at the site of injection in the nude mouse hosts over a more protracted period than the others, we requested and obtained another sample of the same patient’s blood 5.5 months after the first phlebotomy sample. Cells from the second blood sample were transplanted into mice that had not received previous injections of cells.

Phlebotomy was performed with a blood collection tube anticoagulated with lithium heparin (product number 366485; Becton Dickinson and Company, Franklin Lakes, NJ) and transported from the clinic to the laboratory in an ice bath. Under sterile conditions, blood was transferred to a 50-ml beaker. It was then drawn in aliquots up to 5 ml into 5-ml disposable syringes without needles (product number 309603; Becton Dickinson and Company). The orifices of the syringes were wiped to remove any adherent blood. Syringes were allowed to stand in a vertical position with the orifice pointed upward for 30 min at 4°C while RBCs settled. Leaving the sedimented RBCs in the syringe, the pale yellow, leucocyte-rich plasma was then gently expelled by the syringes through bent, U-shaped, 16-gauge needles (product number 305198; Becton Dickinson and Company). Nucleated cells in the leucocyte-rich plasma were counted with a hemocytometer. The plasma was centrifuged at 97 × g for 7.5 min at 4°C. The plasma was discarded, and the cells that had sedimented during centrifugation were resuspended in 1.0 ml of Matrigel (Collaborative Research, Bedford, MA) at 4°C. The Matrigel and the sedimented WBCs, variable numbers of erythrocytes that had not sedimented with the other erythrocytes in the syringes (usually approximately 1.5–3 times as many erythrocytes as nucleated cells), and any circulating tumor cells were drawn into 1-ml syringes. For each patient, two mice were injected s.c. with 0.5 ml of cells in Matrigel adjacent to the scapula through an 18-gauge needle as described previously (5, 6). Mice received 12.5 mg of sustained-release testosterone (Innovative Research of America, Sarasota, FL) s.c., and these sustained-release doses of testosterone were replaced every 3 months as described previously (5–7). Swellings at the sites of injection were measured weekly with calipers.

Cells from Injection Sites. From one mouse that received injections from one patient’s blood cells, the injection site was digested to obtain a cell suspension. The tissue at the injection site was dissociated into a cell suspension. The tissue at the injection site was dissociated by serial digestion of minced tumor with 0.1% Pronase E (VWR Scientific Products, Cleveland, OH) in Joklik’s modification of MEM. This method is described in detail (8) in the only, to our knowledge, published, quantitative comparison of collagenase, Pronase, and trypsin for the dissociation of human primary prostate cancers; it has been used for the dissociation of prostate cancer xenografts (5–7). Cells obtained in suspension were injected into other animals in 0.5 ml of Matrigel as described above.

Histopathology. Animals that received cells from the first 10 patients were killed 6–8 months after cell injection. The remaining animals were killed 4–7 months after cell injection. Sections of the sites of injection and sections of lung, liver, and lymph nodes were examined histologically by two pathologists.

Results

We have found the least dimension to be the most indicative of the growth of prostate cancer cells injected into nude mice in Matrigel (5). As detailed in Table 1 of a previous publication (5), least dimensions of 3 mm are common and least dimensions of 4 mm are less common...
in primary prostate cancer xenografts that fail to grow progressively and become transplantable. Most of the 20 animals (2 mice/patient) that received injections from the first 10 patients exhibited few or no swellings >2 mm in least dimension at the sites of injection, which were monitored weekly over a period of 6–8 months. All of these mice were killed 6–8 months after cell injection. Histological examination of these sites of injection revealed no viable tumor cells but abundant fibrosis and some chronic inflammatory changes. No metastases were identified in any of these mice by gross or histopathological examination.

Based on these data from the first 10 patients, the remaining animals that received injections of nucleated cells from five additional phlebotomies were killed. The sites of injection were similar to those described above; however, to our surprise, poorly differentiated metastatic carcinomas were observed grossly and confirmed histopathologically in the lungs of animals that had received cells from two prostate cancer patients and one patient with colon cancer (Table 1). Only one of the two mice that received cells from a colon cancer patient had a grossly visible lung metastasis that was confirmed histologically; the other mouse lacked any evidence of cancer. Both mice that received cells from one of the prostate cancer patients developed lung metastases; one had grossly visible metastases, and the other had metastases that could only be identified microscopically. As will be elaborated below, both samples of blood cells obtained from a second prostate cancer patient produced pulmonary metastases; the two phlebotomies from this prostate cancer patient were 5.5 months apart.

This prostate cancer patient from whom we received two samples of blood is of particular interest. One of the two mice that received the first injections of cells from this patient developed swellings that vacillated between 2 and 3 mm in least dimension. This mouse, in contrast to most mice, spent the largest proportion of the first 5 months after injection with an injection site that was 3 mm in minimum dimension. Because we had observed a very similar pattern in the first generation of CWR21, a prostate cancer xenograft that was first described in 1993 (5) and is still being passaged serially in our laboratory, the injection site of this mouse was transplanted. Neither of the two original recipients of blood cells had histological evidence of cancer. The transplanted injection site was a very small piece of tissue (0.15 g) and was used entirely as a source of cells in suspension; i.e., none of this small piece of tissue was used for histological examination. A cell suspension was obtained by digesting the tissue fragment from the injection site with Pronase E as described above. The resultant 0.83 million cells were centrifuged and resuspended in 1 ml of Matrigel. Half of this cell suspension in Matrigel was injected into each of two mice. These mice were killed 4.5 months later, when the experiment was terminated. One of the mice had a pulmonary metastasis that was grossly visible and histologically consistent with prostatic carcinoma (Fig. 1).

Because of the frequent swelling of the injection site in the mouse over a period of 5 months, we obtained a second sample of blood from the same patient 5.5 months after the first sample of blood had been received. Cells obtained from the second sample of blood from this patient were injected into two mice as described above. Because no consistent growth was observed at the injection sites of the cells from the first 10 patients after 6–8 months, the experiment was terminated 4.5 months after injection of cells from the blood of the patient from whom two samples were obtained. One of the two mice that received cells from the second phlebotomy sample from this patient showed a pulmonary metastasis (Fig. 1B) that was histologically very similar to the metastasis that resulted from the blood obtained in the first phlebotomy sample (Fig. 1A), which had been obtained 5.5 months earlier.

Table 1 Growth of tumors from patients whose circulating cells were tumorigenic

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Cells injected</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Blood cells, colon cancer patient</td>
<td>2-mm lung metastasis</td>
</tr>
<tr>
<td>2</td>
<td>Blood cells, colon cancer patient</td>
<td>No tumor found</td>
</tr>
<tr>
<td>3</td>
<td>Blood cells, prostate cancer patient 1</td>
<td>4-mm lung metastasis</td>
</tr>
<tr>
<td>4</td>
<td>Blood cells, prostate cancer patient 1</td>
<td>Lung micrometastasis</td>
</tr>
<tr>
<td>5</td>
<td>Blood cells, prostate cancer patient 2, sample 1</td>
<td>No tumor found</td>
</tr>
<tr>
<td>6</td>
<td>Blood cells, prostate cancer patient 2, sample 1</td>
<td>No tumor found; injection site transplanted</td>
</tr>
<tr>
<td>7</td>
<td>Cells from injection site of mouse 6</td>
<td>No tumor found</td>
</tr>
<tr>
<td>8</td>
<td>Cells from injection site of mouse 6</td>
<td>1.5-mm lung metastasis</td>
</tr>
<tr>
<td>9</td>
<td>Blood cells, prostate cancer patient 2, sample 2</td>
<td>No tumor found</td>
</tr>
<tr>
<td>10</td>
<td>Blood cells, prostate cancer patient 2, sample 2</td>
<td>1.5-mm lung metastasis</td>
</tr>
</tbody>
</table>
Discussion

As reviewed in detail (9, 10), the presence of cancer cells in the peripheral circulations of some patients with carcinomas has been known for more than a century (11). Our laboratory reported the purification and culture of malignant melanocytes from patients with metastatic melanoma in 1986 (12). Success in the initiation of cell lines in culture from prostate cancer has generally been <0.1%, as reviewed previously (1, 13). There has been a marked improvement in the successful transplantation of human prostate cancers as xenografts in this decade, as reviewed in detail in Ref. 13. Investigators from several laboratories have succeeded in initiating serially transplantable xenografts from prostate cancers in approximately 5–10% of cases (13).

In 1980, Gittes (14) reviewed attempts to start prostate cancer xenografts and concluded, “The incidence of ‘take’ of prostate cancer has been close to zero.” In 1990, Kleinman et al. (15) published the first of several reports (16, 17) from her laboratory that showed that the use of Matrigel as a vehicle for injection of tumor cells into nude mice greatly increased the tumorigenicity of small cell lung cancer cells and MCF7 breast cancer cells. There have been several subsequent reports from her laboratory that have confirmed this finding in a variety of other cell systems (reviewed in Refs. 1 and 13). In 1991, Pretlow et al. (18) reported that the use of Matrigel as a vehicle for injection reduced the number of cells required for the transplantation of tumors from commonly used prostate cancer cell lines. This reduction in the number of cells required for transplantation was 25,000-fold for PC-3 and 7,000-fold for DU145. In the serial transplantation of the primary human prostate cancer xenografts established in our laboratory (5), we have found that the use of Matrigel as a vehicle for injection has decreased by >1,000-fold the number of cells required for the transplantation of cell suspensions from all of our xenografts. In contrast, the use of Matrigel as a vehicle for injection has not affected the amount of minced xenograft required for successful transplantation of these xenografts.

Injected in Matrigel, PC-3 cells are tumorigenic down to 20 cells/injection. Xenografts from two (CWR22 and CWR91) of the three patients from whose primary prostate cancers we obtained serially transplantable xenografts can be transplanted with <1000 cells. In considering (a) the very small numbers of cells required for the propagation of some prostate cancer cells as xenografts and (b) the fact that Matrigel affects the tumorigenicity of cells in suspension much more than the tumorigenicity of minced tissue, we decided to investigate the possible tumorigenicity of prostate cancer cells circulating in blood. As our first activity, we selected only patients with advanced metastatic disease.

For the experimental development of customized therapy for patients with advanced metastatic cancers, it would seem likely that the characterization of cells that have entered the circulations of these patients would be of considerable interest. Perhaps the molecular characterization of the cancer cells in the circulation would allow the development of new systems of classification that would permit a more sophisticated selection of alternative therapies now in use. The development of xenografts from these cells may allow a more sophisticated testing of therapies in vivo, particularly for patients like those with prostate cancer, who often survive for years after metastases have developed (i.e., for patients whose cancer progression is sufficiently slow to allow xenografts to be started in advance of the time when alternative therapies have to be initiated).

One might hope that there might be clinical or pathological characteristics that would allow one to distinguish the patients whose tumors grew from those patients whose tumors did not grow. There were no such characteristics that we were able to identify. None of the three patients whose tumors grew as xenografts (metastatic to the mouse lungs) had clinical or laboratory evidence of pulmonary or hepatic metastases. The patient with colon cancer had her primary tumor resected in 1996. It recurred in 1998; however, the only tumor identified in 1998 and later, when we obtained blood in 1999, was a tumor in the pelvis near the site of origin of the primary tumor. She and both prostate cancer patients had been examined by computerized tomography in a field that included the liver <6 months before the drawing of blood from which xenografts were obtained. Physical examination, computerized tomography, and blood analyses (for bilirubin levels, alkaline phosphatase activities, and so forth) at the time of phlebotomy and more recently failed to detect any evidence of hepatic abnormalities in any of the three patients. The two prostate cancer patients had metastases to the bone and lymph nodes; however, no other organ systems were involved by metastases that could be detected.

To our knowledge, this is the first reported growth of xenografts from the blood of patients with metastatic carcinomas. The method described here likely could be improved in many ways if the development of xenografts from patients with metastatic cancers were found to provide new avenues for the development and selection of more effective therapies. We used 9.2–21 ml of blood; larger numbers of cancer cells could be obtained from larger samples of blood. It seems likely that leukopheresis could be used to obtain much larger numbers of cells for transplantation. Ghossein et al. (19) have reported the enrichment of prostate cancer cells from the blood of patients with malignant melanoma. Pretlow et al. (20) have reviewed other techniques for the purification of cancer cells. The purification of neoplastic cells from the blood of patients with metastatic cancer may facilitate the generation of xenografts from a larger proportion of patients. The treatment of animals with growth factors either systemically or at the site of the transplanted cells might facilitate the growth of xenografts from cells in the peripheral circulations of patients with metastatic tumors.

References


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