Role of Organ Selectivity in the Determination of Metastatic Patterns of B16 Melanoma

Ian R. Hart and Isaiah J. Fidler

Cancer Metastasis and Treatment Laboratory, National Cancer Institute, Frederick Cancer Research Center, Frederick, Maryland 21701

ABSTRACT

The preferential growth of B16 melanoma metastases in specific organs was studied. Following the i.v. injection of B16 melanoma cells into syngeneic C57BL/6 mice, tumor growths developed in the in situ lungs and in grafts of pulmonary or ovarian tissue implanted either s.c. or i.m. In contrast, neoplastic lesions failed to develop in control grafts of similarly implanted renal tissue or at the site of a surgical trauma. Para-biosis experiments suggested that the growth of the B16 melanoma in ectopic lung or ovary tissue was due to the immediate arrest of circulating neoplastic cells and not to shedding of malignant cells from foci growing in the in situ lungs. Quantitative analysis of tumor cell arrest and distribution using cells labeled with [\(\text{\textsuperscript{125}I}\)]-5-iodo-2'-deoxyuridine indicated that the growth of tumors in the implanted organs was not due to an enhanced initial arrest of B16 cells. No significant differences in immediate tumor cell arrest were detected between implanted fragments of lungs (tumor positive) and kidney (tumor negative) or between organ-bearing and contralateral control limbs. We conclude that the outcome of metastasis is dependent on both tumor cell properties and host factors. This conclusion supports the "seed and soil" hypothesis to explain the nonrandom pattern of cancer metastasis.

INTRODUCTION

Clinical observations of patients with primary tumors of defined histological classification suggest that there is a tendency for metastases to occur in particular distant organs (4). Studies in experimental animal tumor systems have demonstrated that the formation and anatomical location of metastases are determined by both tumor cell properties and multiple host factors (4, 11, 20, 26, 27, 31, 32).

In 1928, Ewing (6) suggested that metastasis is influenced by purely mechanical mechanisms such as anatomical and hemodynamic factors of the vasculature. Although circulatory anatomy may indeed influence the dissemination of many malignant cells, it cannot fully explain the patterns of distribution of numerous tumors. Why organs such as the spleen or skeletal muscle with highly developed vasculature should be frequent sites of metastatic growth is not understood. Similarly, the propensity of certain human and animal tumors for growth in particular organs cannot be accounted for on hemodynamic grounds alone.

To explain this nonrandom pattern of metastasis, Paget (25) in 1889 proposed the "seed and soil" hypothesis, which suggested that some tumor cells find certain organs more fertile an environment for metastatic growth. Later studies of the distribution and fate of radiolabeled tumor cells in experimental systems (7, 10, 12) clearly indicate that tumor cells may indeed reach and be arrested in the microvasculature of many organs. Tumor cell proliferation into visible metastases, however, occurred in some but not all organs. Thus, the arrest of viable tumor cells in a particular organ does not always guarantee subsequent tumor development (12).

Data suggesting that tumor cell properties may determine the outcome of metastasis were reported by Zeidman and Buss (39) who demonstrated that tumor cells from different tumors interact differently with the capillary bed of a given organ. Sugarbaker (34) injected tumor cell suspensions from different types of tumors into the same site in rats and observed that each type established its own pattern of metastases. In separate experiments, Fisher and Fisher (14) demonstrated that tumor cells can traverse different organs at different rates. Strong experimental evidence for the homing to and growth of tumor cells in particular organs was first reported by Kinsey (21) who used the Cloudman melanoma and then by Sugarbaker et al. (35) who used a murine fibrosarcoma. In both studies, neonatal tissue was implanted in the thighs of syngeneic mice. Following the i.a. or s.c. injection of lung-colonizing tumor cells, metastatic foci developed in the in situ lung as well as the grafted lung but not in other grafted organ controls.

What remained unanswered is how certain tumors demonstrate preferential organ metastasis and why most organs in which tumor cells are arrested do not support the growth of metastases. The present report concerns our investigations to determine whether metastatic tumor cells "home" to (i.e., arrest in) specific organs where secondary growth occurs or whether metastatic tumor cells distribute to many organs but grow in only particular sites. Furthermore, we questioned whether mechanical arrest of circulating emboli can explain patterns of metastasis or whether tumor cell:organ affinities are responsible for the growth of metastatic foci.

MATERIALS AND METHODS

Animals. Specific-pathogen-free C57BL/6 mice were obtained from the Animal Production Area of the Frederick Cancer Research Center. Within a single experiment, the mice were age and sex matched.

Tumors and Culture Conditions. B16 melanoma lines B16-F1 (low lung-colonizing potential) and B16-F10 (high lung-colonizing potential) syngeneic to the C57BL/6 mouse were used. All tumor cells were grown in Falcon tissue culture flasks with Eagle's minimal essential medium supplemented with 10% F1 (low lung-colonizing potential) and B16-F10 (high lung-colonizing potential) syngeneic to the C57BL/6 mouse were used. All tumor cells were grown in Falcon tissue culture flasks with Eagle's minimal essential medium supplemented with 10% F1 (low lung-colonizing potential) and B16-F10 (high lung-colonizing potential) syngeneic to the C57BL/6 mouse were used. All tumor cells were grown in Falcon tissue culture flasks with Eagle's minimal essential medium supplemented with 10% F1 (low lung-colonizing potential) and B16-F10 (high lung-colonizing potential) syngeneic to the C57BL/6 mouse were used. All tumor cells were grown in Falcon tissue culture flasks with Eagle's minimal essential medium supplemented with 10% F1 (low lung-colonizing potential) and B16-F10 (high lung-colonizing potential) syngeneic to the C57BL/6 mouse were used. All tumor cells were grown in Falcon tissue culture flasks with Eagle's minimal essential medium supplemented with 10% F1 (low lung-colonizing potential) and B16-F10 (high lung-colonizing potential) syngeneic to the C57BL/6 mouse were used. All tumor cells were grown in Falcon tissue culture flasks with Eagle's minimal essential medium supplemented with 10% F1 (low lung-colonizing potential) and B16-F10 (high lung-colonizing potential) syngeneic to the C57BL/6 mouse were used. All tumor cells were grown in Falcon tissue culture flasks with Eagle's minimal essential medium supplemented with 10% F1 (low lung-colonizing potential) and B16-F10 (high lung-colonizing potential) syngeneic to the C57BL/6 mouse were used. All tumor cells were grown in Falcon tissue culture flasks with Eagle's minimal essential medium supplemented with 10% F1 (low lung-colonizing potential) and B16-F10 (high lung-colonizing potential) syngeneic to the C57BL/6 mouse were used. All tumor cells were grown in Falcon tissue culture flasks with Eagle's minimal essential medium supplemented with 10% F1 (low lung-colonizing potential) and B16-F10 (high lung-colonizing potential) syngeneic to the C57BL/6 mouse were used. All tumor cells were grown in Falcon tissue culture flasks with Eagle's minimal essential medium supplemented with 10% F1 (low lung-colonizing potential) and B16-F10 (high lung-colonizing potential) syngeneic to the C57BL/6 mouse were used. All tumor cells were grown in Falcon tissue culture flasks with Eagle's minimal essential medium supplemented with 10% F1 (low lung-colonizing potential) and B16-F10 (high lung-colonizing potential) syngeneic to the C57BL/6 mouse were used. All tumor cells were grown in Falcon tissue culture flasks with Eagle's minimal essential medium supplemented with 10% F1 (low lung-colonizing potential) and B16-F10 (high lung-colonizing potential) syngeneic to the C57BL/6 mouse were used. All tumor cells were grown in Falcon tissue culture flasks with Eagle's minimal essential medium supplemented with 10% F1 (low lung-colonizing potential) and B16-F10 (high lung-colonizing potential) syngeneic to the C57BL/6 mouse were used. All tumor cells were grown in Falcon tissue culture flasks with Eagle's minimal essential medium supplemented with 10% F1 (low lung-colonizing potential) and B16-F10 (high lung-colonizing potential) syngeneic to the C57BL/6 mouse were used. All tumor cells were grown in Falcon tissue culture flasks with Eagle's minimal essential medium supplemented with 10% F1 (low lung-colonizing potential) and B16-F10 (high lung-colonizing potential) syngeneic to the C57BL/6 mouse were used. All tumor cells were grown in Falcon tissue culture flasks with Eagle's minimal essential medium supplemented with 10% F1 (low lung-colonizing potential) and B16-F10 (high lung-colonizing potential) syngeneic to the C57BL/6 mouse were used. All tumor cells were grown in Falcon tissue culture flasks with Eagle's minimal essential medium supplemented with 10% F1 (low lung-colonizing potential) and B16-F10 (high lung-colonizing potential) syngeneic to the C57BL/6 mouse were used. All tumor cells were grown in Falcon tissue culture flasks with Eagle's minimal essential medium supplemented with 10% F1 (low lung-colonizing potential) and B16-F10 (high lung-colonizing potential) syngeneic to the C57BL/6 mouse were used. All tumor cells were grown in Falcon tissue culture flasks with Eagle's minimal essential medium supplemented with 10%
fetal bovine serum, sodium pyruvate, nonessential amino acids, L-glutamine, and 2-fold-concentrated vitamin solution as described elsewhere (10). The tumor cell lines were tested for and found free of Mycoplasma and the following murine viruses: reovirus type 3; pneumonia virus of mice; K virus; Theiler's encephalitis virus; Sendai virus; minute virus of mice; mouse adenovirus; mouse hepatitis virus; lymphocytic choriomeningitis virus; ectromelia virus; and lactate dehydrogenase virus (Microbiological Associates, Walkersville, Md.).

For in vivo studies, we harvested the tumor cells from subconfluent cultures in exponential growth phase by overlaying the cells with a thin layer of 0.25% trypsin-0.02% EDTA for 1 min. The flask was tapped sharply to dislodge the cells, and Eagle's minimal essential medium supplemented with 10% fetal bovine serum, sodium pyruvate, nonessential amino acids, L-glutamine, and 2-fold-concentrated vitamin solution was added immediately. The cells were then washed and resuspended in HBSS. Tumor cell viability was about 95% based on the ability of the cells to exclude trypan blue. Only suspensions composed of single cells were used for i.v. injection into animals.

Tumor Cell Dissemination and Growth following i.v. Injection. Melanoma cells were harvested as described above, and their number was adjusted to $2.5 \times 10^7$ viable cells/ml HBSS. Viability was assessed by trypan blue exclusion, and only suspensions with $>95\%$ cell viability were used. Mice received inoculations of $5 \times 10^7$ cells in 0.2 ml HBSS via the tail vein. Three weeks after receiving injection, mice were killed and autopsied. Extrapulmonary tumors were noted, and the transplant sites (see below) were fixed and processed for histology. Lungs were removed and rinsed in water, and pulmonary nodules were counted with the aid of a dissecting microscope.

Parabiosis. Six- to 8-week-old C57BL/6 mice received i.v. injection of $5 \times 10^4$ viable B16-F10 cells in 0.2 ml HBSS 7 days prior to parabiosis. Radiolabeled distribution studies conducted in this laboratory have shown that 7 days after the i.v. injection of tumor cells, there are no detectable tumor cells in the circulation, but extravasation into the lung parenchyma has occurred (7, 10, 12). Tumor-bearing mice were joined parabiotically to normal controls using the modified technique of Eichwald et al. (5) as we have fully described elsewhere (13). Two to 3 weeks after i.v. tumor cell injection, some of the parabionts were killed and autopsied. In other experiments, the parabionts were separated, and the parabionts not given injection were allowed to survive 2 to 3 additional weeks and then autopsied.

Skin Grafting. Tumor-bearing mice (7 days following i.v. injection of B16 cells) were anesthetized by i.p. injection of sodium barbital. The ventral abdomen was shaved, and 2 curving incisions approximately 2 cm long were made through the skin. The full thickness of elliptical skin isolated by these incisions was removed by blunt dissection and stretched, and a 1-cm-diameter circle of tissue was punched out using a cork borer. This portion of skin was placed subcutis down on the exposed s.c. tissue (hair follicles in the opposite direction to those of the recipient) and kept in place with an adhesive bandage fastened by a wound clip. The mice were allowed to recover and housed 2/cage until killed 2 weeks later.

Organ Transplantation. Newborn C57BL/6 mice less than 24 hr old were killed by decapitation. The lungs and kidneys were removed immediately and placed into cold HBSS. Kidneys were kept intact, but the lungs were divided into fragments of approximately 1 cu mm. In one experiment, intact ovaries were removed from 8-week-old C57BL/6 mice from transplantation; these organs were also kept intact. All organs were transplanted within 60 min. Recipient mice (6- to 8-week-old male C57BL/6) were anesthetized with methoxyflurane (Metofane; Pitman-Moore, Inc., Washington Crossing, N. J.), and the hind leg was shaved. An incision was made through the skin and into the body of the quadriceps femoris muscle. A fragment of the appropriate organ was placed with forceps into the muscle mass, and the skin wound was closed with 2 to 3 stainless steel, 9-mm wound clips. In some experiments detailed under “Results,” organ fragments were deposited s.c. in the thigh with a trocar. Control surgical procedures (trauma) were as above except that organ fragments were not deposited in the wound. Animals were allowed a 2- to 3-week recovery prior to their receiving i.v. an injection of tumor cells since it has been our experience that wound repair and organ vascularization have occurred by this time.

Experimental Procedure. Mice receiving grafts of a variety of organ fragments were given an injection i.v. of $5 \times 10^4$ viable melanoma cells. Three weeks following injection, the mice were killed and autopsied. Metastases in the in situ lungs and other organs were determined under a dissecting microscope. Metastases in the grafted organ site or site of surgical trauma were determined with the aid of a dissecting microscope and always were confirmed histologically.

Quantitative Analysis of Tumor Cell Arrest. For in vivo localization studies, tumor cells prelabeled in vitro with $[^{125}I]$-IdUrd were injected into the tail vein of recipient mice. The technique of labeling tumor cell DNA with $[^{125}I]$IdUrd has been described previously (7). At intervals from 10 min to 1 day postinjection, mice were killed. Five mice/group were evaluated at each time point. Lungs and liver were collected from each mouse and washed for 48 hr in 2 changes of 70% ethanol to remove practically all ethanol-soluble $^{125}I$. The remaining ethanol-insoluble radioactivity is associated with DNA of tumor cells viable at time of kill (6). The hind limbs were separated at the pelvic girdle and monitored separately in a $\gamma$-counter.

Statistical Analysis. Data were analyzed by the Student’s 2-tailed $t$ test or by the 2-tailed Mann-Whitney $U$ test as detailed in “Results.”

RESULTS

Tumor Growth in Ectopic Organs. The data from 3 separate experiments in which various organs were implanted in the thigh muscle are summarized in Table 1. Following the i.v. injection of B16-F10 cells, 20 of 28 animals developed experimental metastases in the grafted pulmonary tissue. In contrast, only 4 of 28 animals developed tumor growths at the site of a similarly transplanted kidney fragment. In one experiment with ovaries implanted in the thigh, 7 of 10 animals so treated...
developed tumor growth at this site. Tumor growth was determined by visual observation but was always confirmed histologically. The histology of grafted organs and tumortastases is shown in Figs. 1 to 4. Identification of implanted organs was surprisingly uncomplicated even 5 weeks after transplantation. Lung fragments were identified by the presence of small bronchioles, some of which were still lined by columnar epithelium. Kidney grafts contained glomeruli in various stages of degeneration, and grafted ovaries were identified by the presence of ova (Fig. 1).

The possibility that traumatic injury induced during incision of the thigh was responsible for tumor cell lodgment and growth (15) was ruled out by 3 different lines of experimental evidence. The data are summarized in Table 2. (a) Traumatic implantation, where the thigh muscle was incised and the ectopic tissue was embedded within the muscle, produced no more neoplastic lesions at the graft site than when the tissue was merely deposited s.c. with a trocar (i.e., atraumatic implantation). Tumors developed at the implantation sites of both lung fragments (traumatic, 8 of 10; atraumatic, 7 of 8) and ovary (traumatic, 7 of 10; atraumatic, 7 of 9) but not to any great degree at the site of the implanted kidney fragments (traumatic, 2 of 10; atraumatic, 0 of 10). (b) In some experiments, the mice were subjected to surgery, but no organ implantation took place (trauma controls). Tumors developed in only 1 of 10 such mice. (c) Tumor growth in ectopic organs was specific and not due to host defect per se. This was shown by experiments in which different organs were implanted in the opposite hind legs. Tumors developed at 6 of 10 of the pulmonary grafts, but none developed at the contralateral site implanted with kidney fragments. Three of 10 animals receiving implantations at the same site of a combination of kidney and lung fragments developed tumors at the implantation area.

To rule out the possibility that the implanted lung tissue nonspecifically supports the growth of any injected tumor, we performed the following control experiment. The M5076 granulosa cell tumor (obtained from Dr. D. Griswold, Southern Research Institute) is known to metastasize in syngeneic C57BL/6 mice following i.v. or s.c. implantation (33). The visceral organs (ovaries, liver, spleen, and kidneys) are common sites of tumor growth, but even after the i.v. injection of tumor cell aggregates (10 to 20 cells/clump), no tumor foci have been found in the lungs. C57BL/6 mice bearing pulmonary grafts as described in "Materials and Methods" were given an injection i.v. of 5 x 10^4 viable M5076 cells. Although tumor incidence and occurrence in the visceral organs were unaltered, only 1 of 10 animals developed M5076 tumors at the lung graft site as compared with 7 of 10 animals receiving injection of the B16-F10 melanoma. However, 5 of 10 animals developed M5076 tumors at the site of ovary grafts, showing that this tumor is capable of growth in ectopically maintained organs.

The incidence of melanoma metastasis observed in the in situ lungs of mice given implantation of either kidney or lung fragments is shown in Table 3. Mice that received injection of B16-F1 cells had a median of 9 lung tumor nodules (range, 1 to 15). In contrast, mice that received injection of equal numbers of viable B16-F10 cells had a median of 91 lung tumor nodules (range, 35 to 124). These significant differences (p < 0.001) agree with our previous results (8, 9, 12, 13) and those of others (23, 24, 28).

Parabiosis and Skin Grafting. The formation of tumor foci at the transplanted organ site could have been due to the arrest and growth of tumor cells immediately following i.v. injection, i.e., "initial metastases." Alternatively, tumor cells injected i.v. could have been arrested in a given in situ organ (e.g., lungs), developed there, and then shed tumor cells into the circulation to be arrested at other sites to form subsequent "secondary metastases." To clarify these possibilities, we performed several experiments with parabiotically joined mice. The results of some of these experiments are given in Table 4. Two weeks after untreated mice ("guests") had joined parabiotically to tumor-bearing animals ("hosts"), there was no evidence of any tumor growth in the guest animals. However, when another series of animals was allowed to survive for an additional 2

Table 1
Growth of i.v. disseminated B16-F10 cells in ectopic organs

<table>
<thead>
<tr>
<th>Implanted organ</th>
<th>No. of mice with tumor growing in transplant site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td>2/8</td>
</tr>
<tr>
<td>Lung</td>
<td>7/10</td>
</tr>
<tr>
<td>Ovary</td>
<td>7/10</td>
</tr>
</tbody>
</table>

*M57BL/6 mice with organ fragment implanted into an incised thigh muscle. The surgical site was closed, and 2 weeks later the mice received i.v. injections of 5 x 10^4 viable B16-F10 cells. Tumor growth was confirmed grossly and histologically 2 to 3 weeks after i.v. injection.

Number in parentheses, range.

Table 2
Effect of surgical trauma on growth of B16-F10 cells at the site of organ transplantation

<table>
<thead>
<tr>
<th>Implanted organ</th>
<th>Traumatic implantation</th>
<th>Atraumatic implantation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td>2/10</td>
<td>0/10</td>
</tr>
<tr>
<td>Lung</td>
<td>8/10</td>
<td>7/8</td>
</tr>
<tr>
<td>Ovary</td>
<td>7/10</td>
<td>7/9</td>
</tr>
<tr>
<td>Left leg, kidney</td>
<td>0/10</td>
<td></td>
</tr>
<tr>
<td>Right leg, lung</td>
<td>6/10</td>
<td></td>
</tr>
<tr>
<td>Kidney and lung</td>
<td>3/10</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>1/10</td>
<td></td>
</tr>
</tbody>
</table>

*See Table 1, Footnote a.

Table 3
Effect of in situ lung tumor burden on the growth of melanoma cells at the site of organ transplantation

<table>
<thead>
<tr>
<th>Cell line</th>
<th>No. of mice with tumor at transplant site</th>
<th>Median no. of lung nodules in the in situ lung</th>
</tr>
</thead>
<tbody>
<tr>
<td>B16-F1</td>
<td>7/9</td>
<td>9 (1-15)</td>
</tr>
<tr>
<td>B16-F10</td>
<td>5/10</td>
<td>91 (35-124)</td>
</tr>
</tbody>
</table>

*Ten C57BL/6 mice/group received injections i.v. of 5 x 10^4 viable cells. The mice were killed 3 weeks later. The number of metastases was determined with a dissecting microscope.

*a The number of lung metastases produced by B16-F10 cells differed significantly from that of B16-F1 cells (p < 0.001 [Mann-Whitney U test]).

Numbers in parentheses, range.

I. J. Fidler and I. R. Hart, unpublished observations.
weeks (after separation), 6 of 15 (40%) mice developed lung nodules, and 9 of 15 or 60% had tumor growth at the wound (parabiosis) site.

The frequent incidence of tumor growth at the surgical site of parabiosis raised the question of whether tumor growths in the mice joined in parabiosis were due to dissemination of tumor from established neoplastic foci, i.e., metastases of metastases (18), or whether the surgical trauma had "awoken" dormant cells that had lodged in the skin after the initial arrest of i.v. injected cells, i.e., 7 days prior to surgery, followed by local spread to the "guest" parabionts. To distinguish between these possibilities, we obtained segments of skin from tumor-bearing mice and grafted those onto normal syngeneic recipients. Nine of 9 skin grafts were accepted, but no tumors developed at the graft site of the recipient not receiving injections or in the recipient's lung even 6 to 10 weeks after grafting. At the same time, in 2 of 8 donor (tumor-bearing) mice, tumors developed at the skin graft donor site (wound) (Table 5).

**Kinetics of Initial Tumor Cell Arrest.** To determine whether tumor growth at ectopically implanted organs was due to organ-specific arrest of i.v. injected cells, we measured cell distribution. Data from one representative experiment (of several) are shown in Table 6. By 10 min after injection of 5 × 10⁴ [¹²⁵I]-IdUrd-labeled B16-F10 cells, no more than 0.8% of the total number of injected cells was lodged in the hind legs. No significant difference could be detected between the number of cells lodged in limbs bearing renal or pulmonary grafts at the 3 selected time points of 10 and 60 min and 1 day (Student's 2-tailed t test). Equally, no significant differences in tumor cell arrest were detected among the different implanted organs or between operated and nonoperated legs.

**Tumor Cell Dissemination and Growth following i.v. Injection.** Data drawn together retrospectively from several experiments are presented in Table 7. In this set of experiments, the median number of lung nodules produced 3 weeks following i.v. injection of 5 × 10⁴ viable B16-F10 cells was 86 in male mice as compared to 136 in female mice (p ≤ 0.001 [Mann-Whitney U test]). Of interest is the association of extrapulmonary metastases with the gonads. In males, 23 of 107 animals (21.5%) had grossly evident testicular tumors, whereas 32 of 47 (68%) female mice displayed grossly apparent ovarian tumor growths. These tumors, however, were smaller than those growing in the lungs. In contrast to this high incidence of gonadal involvement, only 10% of males and 13% of females had extrapulmonary lesions in organs other than the testes or ovaries. The kidney was involved in 8% of the males and 2% of the females examined. About 1% of mice of either gender developed liver metastases. These results were obtained from mice killed 3 weeks after tumor cell injection. No extrapulmonary tumors were observed in mice killed 2 weeks after tumor cell inoculation, agreeing with earlier published reports (13, 23).

**DISCUSSION**

The data presented here confirm similar observations made by Kinsey (21) and Sugarbaker et al. (35), clearly demonstrate the organ-specific nature of metastatic spread of the B16 melanoma, and suggest some of the mechanisms responsible for such nonrandom distribution. Following the i.v. injection of B16

---

**Table 4**

Differentiation between "initial" and "secondary" metastasis using parabiosed C57BL/6 mice

| Protocol | Hosts | | | Guests |
|---|---|---|---|
| 1. Hosts received injections i.v. of 5 × 10⁴ viable cells. One wk later, they were parabiotically joined to guests and 2 wk later autopsied. | No. of mice bearing tumors | Median no. of nodules | No. of mice bearing tumors | Median no. of nodules |
| | 10/10⁴ | 81 (31–300) | 0/10 |
| 2. As above, except that 2 wk after parabiosis, the mice were separated, and guests were allowed to survive a further 10 days. | 6/15⁵ | 9/15⁵ |

---

**Table 5**

Results of skin grafting from tumor-bearing to control animals

<table>
<thead>
<tr>
<th>Animals</th>
<th>No. of animals with tumor at wound</th>
<th>No. of animals with lung nodules</th>
<th>Median no. of lung nodules</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin graft recipients</td>
<td>0/9</td>
<td>0/9</td>
<td></td>
</tr>
<tr>
<td>Skin donors (tumor bearers)</td>
<td>2/9</td>
<td>8/8</td>
<td>101 (47–134)⁰</td>
</tr>
</tbody>
</table>

---

**Table 6**

Arrest of [¹²⁵I]IdUrd-labeled B16-F10 cells at transplant site

<table>
<thead>
<tr>
<th>Time</th>
<th>Transplant-beariing limb</th>
<th>Control limb</th>
<th>Transplant-bearing limb</th>
<th>Control limb</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 min</td>
<td>312 ± 32</td>
<td>278 ± 86</td>
<td>360 ± 45</td>
<td>396 ± 76</td>
</tr>
<tr>
<td>60 min</td>
<td>324 ± 38</td>
<td>259 ± 56</td>
<td>400 ± 141</td>
<td>331 ± 90</td>
</tr>
<tr>
<td>24 hr</td>
<td>159 ± 64</td>
<td>107 ± 14</td>
<td>168 ± 29</td>
<td>100 ± 38</td>
</tr>
</tbody>
</table>

---

* Mean number of labeled tumor cells (5 animals/time interval).
melanoma into syngeneic C57BL/6 mice, experimental metastatic lesions developed in grafts of pulmonary or ovarian tissue placed either s.c. or i.m. In contrast, neoplastic lesions failed to develop in control grafts of similarly implanted renal tissue or at the site of a surgical trauma (Tables 1 and 2). Since renal grafts appeared to survive and vascularize as well as lung or ovarian grafts (Figs. 1 to 4), the explanation for this selective pattern of tumor growth is more complex than simple tissue damage per se creating a suitable microenvironment for tumor cell proliferation (15).

Proctor et al. (30) reported the effect of endocrine factors on the growth and spread of the B16 melanoma. This tumor grew more slowly and metastasized less frequently in female than in male mice following i.m. implantation. These differences could be abrogated by the surgical ablation of female hormone function (30). We too have demonstrated that there is an association between the gender of the recipient and B16 melanoma growth and that preferential metastasis to the gonads may suggest an endocrine role in tumor spread (Table 6). Female mice developed significantly more lung nodules than did male mice. These findings are at variance with the observations of Proctor et al. (30) and with the reported clinical manifestations of melanoma in humans (3, 38). These apparently contradictory data, however, might be due to the different routes of tumor cell administration. In our studies, tumor cells were introduced directly into the circulation, thus bypassing the need for primary tumor growth and entry into the circulation. Whatever the cause of the predilection for in situ ovary shown by i.v. injected B16-F10 cells, it is maintained when the ovarian tissue is grafted into ectopic sites.

We needed to determine whether the growth of tumors at graft sites was the consequence of initial cell spread of neoplastic cells following i.v. injection (primary spread) or whether it was a result of metastasis from established lung colonies (secondary spread). Radiolabeled distribution studies have shown that by 1 week postinjection, B16-F10 cells are eliminated from the blood stream and are lodged in the lungs (7, 12) where they grow into tumor colonies (10). These former studies strongly suggest that the tumor-bearing animals used in the parabiosis experiments had no circulating tumor cells at the time of surgery. Parabiotically joining tumor bearers to normal control animals demonstrated that it was unlikely that growths at ectopic tissue implants in the time period of our experiments were the result of secondary spread. Two weeks after parabiosis, none of the "guest" mice had visible metastatic lesions though tissue-implanted animals had grossly obvious tumors at the surgical sites by this time. That metastases do metastasize was shown here by allowing separated "guests" to survive for an additional 2 weeks. At the end of this period, 60% of these animals had visible tumor deposits. Metastases have been shown to metastasize in some other (18) but not all (35) rodent tumor systems. To demonstrate that the growth of tumors in "guest" animals was not the result of surgical trauma which can "awaken" dormant cells lodged in the skin, we grafted skin from tumor-bearing mice onto normal mice. No tumors developed at the skin graft sites in recipient animals in spite of a 100% acceptance and take of the skin grafts. It was, therefore, unlikely that tumor growth in "guest" parabionts was the result of stimulated dormant cells. Further support for the hypothesis that development of tumors at the site of organ implants was the result of primary tumor cell spread versus overflow from the lung foci was provided by the experiment involving B16-F1 cells (low lung colonization) (Table 3). Although they had one-tenth the tumor burden of those mice receiving B16-F10 cell injections (as assessed by lung nodule counts), 7 of 10 animals bearing lung implants developed tumor growths at the graft site compared with 5 of 10 mice receiving B16-F10 cell injections.

Metastatic lesions at the site of organ implants appear to have developed as a result of initial cell spread. The B16-F10 variant was selected on the basis of its lung-colonizing ability (8), and its propensity for lodgment in pulmonary tissue has been well documented (12). Nicolson and Winkelhake (24) showed that in vitro, B16-F10 cells formed homotypic aggregates with dissociated lung tissue to a greater extent than did B16-F1 cells (parental line). Could such an affinity for pulmonary tissue cause an enhanced arrest of B16-F10 cells at lung grafts and solely account for the data presented here? The results from the B16-F1 experiment could not be explained if such a mechanism only was responsible for the observed metastatic patterns. Moreover, no significant quantitative differences in radiolabeled tumor cell arrest were detected between the various implanted organs or between graft bearer and control limbs, with the exception of the kidney implants where, at 24 hr, more cells were held in the transplant-bearing limb (p < 0.01) than in the contralateral limb. Tumor growths, however, did not develop in kidney implants.

A small number of control animals developed tumors at the sites of surgical trauma or kidney implants. Similar appearance of visible tumors at wound sites was noted in the parabiotic mice and skin graft donors. Although the clinical relevance of iatrogenic local recurrence may be debatable (36), the experimental development of tumor growth at sites of tissue damage has long been established (15). Initial radiolabeled cell distribution studies suggest that the vascularization of the various tissue implants is very comparable. Based on histological ap-
pearance, both lung and renal tissues are well vascularized, but whether subsequent vascularization is different, leading to enhanced or diminished tumor growth, remains to be determined.

These findings illustrate the difficulty encountered in past studies in defining tumor cell "homing." The B16 melanoma appears to "home" to lung and ovarian tissue in that grossly obvious tumor masses, albeit of different colony size, develop in in situ and transplanted organs following i.v. injection of tumor cells. However, it is not apparent that there is an increased initial lodgment of tumor cells in these sites but rather that there are preferential growth and multiplication of tumor cells in these environments. The use of the term "homing" in the context of tumor dissemination must refer to both the localization and subsequent growth of tumor cells at specific organ sites.

Tumor cell properties certainly contribute to organ arrest patterns. Organ-specific variant lines have been selected from parent tumor populations 

ferences

References

Fig. 1. Adult ovary 6 weeks after implantation into thigh of adult C57BL/6 mouse. H & E, × 125.

Fig. 2. Portion of neonatal lung tissue 6 weeks after implantation into thigh of adult C57BL/6 mouse and 3 weeks after i.v. injection of B16 melanoma cells into recipient animal. Columnar epithelium lining bronchi are still apparent. Arrow, focus of melanoma growing in implant. H & E, × 125.

Fig. 3. Portion of neonatal kidney 6 weeks after implantation into the thigh muscle of adult C57BL/6 mouse. Despite degeneration, the glomeruli are still identifiable. H & E, × 125.

Fig. 4. Ovarian implant almost totally replaced by melanoma. The tumor is growing within the ectopic ovary and has not penetrated into the surrounding skeletal muscle. H & E, × 125.
Role of Organ Selectivity in the Determination of Metastatic Patterns of B16 Melanoma

Ian R. Hart and Isaiah J. Fidler


Updated version Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/40/7/2281

E-mail alerts Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.