Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 4661–4665, May 1995
Medical Sciences

Species-specific metastasis of human tumor cells in the severe combined immunodeficiency mouse engrafted with human tissue

EMMA SHTIVELMAN AND REIKO NAMIKAWA*

New Enterprise Research Division, Systemix Inc., 1501 California Avenue, Palo Alto, CA 94304

Communicated by Irving L. Weissman, Stanford University School of Medicine, Stanford, CA, January 20, 1995

ABSTRACT **We have attempted to model human metastatic disease by implanting human target organs into the immunodeficient C.B-17 *scid/scid* (severe combined immunodeficiency; SCID) mouse, creating SCID-hu mice. Preferential metastasis to implants of human fetal lung and human fetal bone marrow occurred after i.v. injection of human small cell lung cancer (SCLC) cells into SCID-hu mice; the homologous mouse organs were spared. Clinically more aggressive variant SCLC cells metastasized more efficiently to human fetal lung implants than did cells from classic SCLC. Metastasis of variant SCLC to human fetal bone marrow was enhanced in SCID-hu mice exposed to γ-irradiation or to interleukin 1α. These data indicate that the SCID-hu mice may provide a model in which to study species- and tissue-specific steps of the human metastatic process.**

Research on tumor metastasis relies heavily on the availability of experimental *in vivo* metastasis models. However, understanding of the mechanisms of metastasis of human tumors has been hampered by the fact that many malignant human tumors are poorly tumorigenic and/or metastatic after inoculation into immunodeficient rodents (1, 2). The introduction of orthotopic implantation of human tumor cells into homologous mouse organs has significantly improved the outcome of metastasis assays (3–6). However, some human tumors apparently require a specific human tissue microenvironment to reproduce their clinical growth and invasion patterns in experimental *in vivo* models. Thus, growth of primary human T-cell lymphomas is greatly enhanced in human lymphoid microenvironment in severe combined immunodeficiency mice engrafted with human tissue (SCID-hu mice) (7). Primary human myeloid leukemias grow selectively within the human bone marrow implanted in SCID mice (8). Human melanoma cells faithfully reproduce the clinical patterns of growth and invasion only when injected into human skin grafts implanted in SCID mice (9).

The tissue-specificity of metastases associated with different human malignancies has been difficult to reproduce in the existing *in vivo* metastasis models. The majority of human tumor cells that demonstrate metastatic behavior in mouse models usually generate pulmonary metastatic lesions. We hypothesized that the lack of metastases or lack of the tissue-specificity of metastasis of malignant human cells in immunodeficient rodents might be, at least in part, due to the possibility that the mechanisms governing the establishment of tissue-specific metastases are also species-specific. As a consequence, experimental metastasis models that involve passage of malignant human cells into immunodeficient rodents may either fail to detect metastatic potential or, alternatively, select for the nonrepresentative outgrowth of human cell variants that can interact with murine organ microenvironments.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

We set out to test the hypothesis that certain human tumor cells that are unable to lodge and/or grow in mouse organs, and thus are scored as negative in existing experimental metastasis models, might prove to be metastatic to human organs implanted within the SCID mouse (10, 11). To this end, we have constructed chimeric SCID-hu mice engrafted with human fetal lung (HFL) and bone marrow (HFBM). To explore the potential of the SCID-hu model for the analysis of metastatic human tumor cells, we have used cell lines derived from small cell lung carcinoma (SCLC), a highly metastatic malignancy of human lung (12). In spite of the extremely aggressive nature of SCLC, no *in vivo* metastasis models for this malignancy have been described. We show here that SCLC cells, when introduced into SCID-hu mice, metastasize preferentially to the engrafted human tissues.

MATERIALS AND METHODS

Cell Culture and Reagents. SCLC cell lines NCI-N417, NCI-H82, NCI-H446, NCI-H146, NCI-H345, and NCI-H69 (13) were obtained from the American Type Culture Collection. SCLC lines ACC-LC-51, ACC-LC-52, and ACC-LC-60 were obtained from T. Takahashi (Aichi Cancer Center, Nagoya, Japan) (14). All cell lines were cultured in RPMI 1640 medium/10% fetal calf serum. Cyclophosphamide was purchased from Sigma. Recombinant human interleukin 1α (IL-1α) was purchased from R & D Systems.

SCID-hu Mice. Homozygous C.B-17 *scid/scid* mice were used at the age of 6–8 weeks. Methoxyflurane anesthesia was used during all operative procedures. The experimental protocols were approved by the Animal Care Committee of Systemix. The human fetal tissues were obtained with informed consent according to regulations issued by each state and by the federal government. For construction of SCID mice implanted with HFL tissue (SCID-hu-L), HFLs of 18–22 gestational weeks were cut into fragments of ≈2 × 2 × 2 mm and surgically implanted into the mouse fourth mammary fat pads and in some cases also under the left kidney capsule. Construction of SCID mice implanted with human fetal intestinal tissues was done in a manner identical to that of SCID-hu-L. We have not observed any signs of inflammation or granulation in the lung or intestine grafts or in the surrounding murine tissues (Fig. 1 *B–D*). Engraftment of SCID mice with HFBM (SCID-hu-BM) was described in detail (15). Briefly, human fetal femurs and tibias at 18–22 gestational weeks were cut into four fragments, which were implanted individually at one or two subcutaneous sites into SCID mice. In some experiments, backbones from newborn SCID mice were implanted into mammary fat pads of SCID mice.

Abbreviations: SCID mice, severe combined immunodeficiency mice; HFL, human fetal lung; HFBM, human fetal bone marrow; SCID-hu-L, SCID mice engrafted with HFL; SCID-hu-BM, SCID mice engrafted with HFBM; SCLC, small cell lung carcinoma; IL-1α, interleukin 1α; v-SCLC, variant-SCLC; c-SCLC, classic SCLC.

*Present address: DNAX Research Institute, 901 California Avenue, Palo Alto, CA 94304.

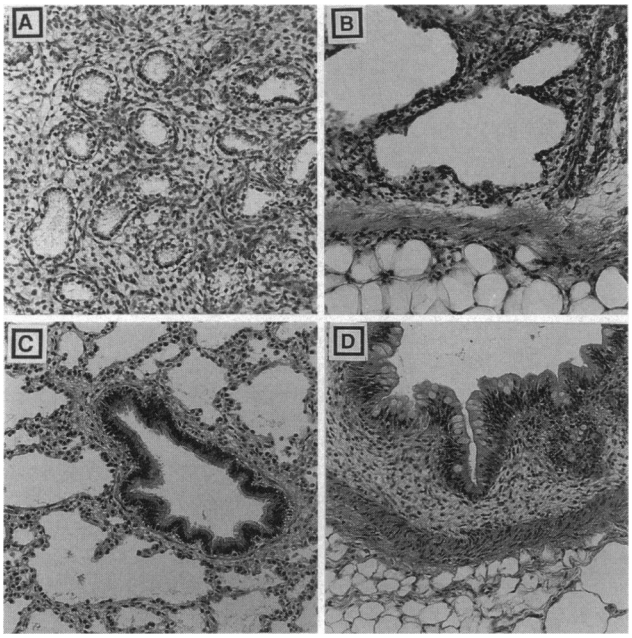

FIG. 1. Engraftment of HFL and intestine tissues in SCID mice. (*A*) HFL tissue, week 18 of gestation (original magnification ×110). (*B*) HFL graft at 4 weeks after implantation bordered by the murine mammary fat tissue (×110). (*C*) HFL graft at 12 weeks after implantation with a bronchiole present (×110). (*D*) Graft of human fetal intestine at 6 weeks after implantation bordered by mammary fat pad tissue. Hematoxylin/eosin staining.

Irradiation. Whole-body irradiation at single doses of 2 or 3 Gy was done with a Gamma Cell 40 irradiator from a ^{137}Cs source at a dose rate of 1.1 Gy/min with 30% attenuation.

***In Vivo* Metastasis Assays.** SCLC cells grown *in vitro* as suspension cultures were harvested by centrifugation, resuspended in Hanks' balanced saline solution (HBSS), assessed for cell number and viability, and injected into SCID-hu mice via lateral tail vein (experimental metastasis assay). For spontaneous metastasis assay cells were injected directly into one of the HFL grafts through a small incision in the skin.

Histology. Fragments of human grafts, murine internal organs (lungs, liver, spleen, adrenals, and sometimes additional organs), backbones, and sternums were dissected and fixed in buffered 20% (vol/vol) formalin. Bone tissues were treated with decalcifying solution (Baxter Scientific Products, McGaw Park, IL). After paraffin embedding, 4-μm sections were cut and stained with hematoxylin/eosin.

RESULTS

Metastasis of SCLC to Human Lung Tissue in SCID-hu-L. Fragments of HFL implanted into mammary fat pads or under renal capsules of SCID mice maintained normal morphology of lung tissues for at least 16 weeks after implantation (Fig. 1). Engraftment was accompanied by an increase in the size of the implants and by morphological changes consistent with the development of a more differentiated phenotype. Thus, the canalicular development phase characteristic for the fetal lung at weeks 16 to ≈24 of gestation (Fig. 1*A*) was changed to the more mature alveolar phase in the grafts implanted for 3–4 weeks and longer (Fig. 1 *B* and *C*).

SCID-hu-L were used for metastasis assays at 4 to 10 weeks after implantation. SCLC cell lines in this study were derived either from the more aggressive and refractory variant SCLC (v-SCLC) subtype (N417, H82, and H446) or from the classic SCLC (c-SCLC) subtype (H146, H69, H345, ACC-LC-52, ACC-LC-51, and ACC-LC-60). Four to five weeks after i.v. injection, v-SCLC lines N417 and H82 induced easily palpable tumors within the implanted HFL tissues of 80–100% of injected mice (Table 1). In most affected mice, more than one of either two or three human lung implants supported tumor growth (Table 1). Frequently more than one tumor developed within the same HFL graft (Fig. 2*A*). v-SCLC H446 metastasized to HFL with a somewhat lower frequency and a longer latent period (Table 1). Thus, metastasis of v-SCLC lines to HFL occurs with high efficiency. Intravenous injection of as few as 10^4 N417 cells into SCID-hu-L resulted in development of tumors in HFL grafts of 60% of mice (data not shown). Tumor cells retrieved from HFL tumors induced by v-SCLC cell lines and re-established *in vitro* were also metastatic to HFL grafts in SCID-hu-L (Table 1).

The morphology of the tumors was typical of v-SCLC: polygonal cells with a relatively scant cytoplasm were seen to be arranged in sheets supported by a very thin stroma. Mitotic figures were frequent, as were extensive areas of central necrosis (Fig. 2 *B* and *C*). Immunohistochemical staining of tumor sections (data not shown) revealed that tumors of N417 origin in HFL implants expressed neural-cellular adhesion molecule (N-CAM) and synaptophysin and were weakly pos-

Table 1. Experimental metastasis of SCLC to HFL implants in SCID-hu-L

Injected cells	Cells injected per mouse, no.	Latency, weeks	Mice with tumors in HFL/ mice injected	Tumors/total HFL implants	Mice with tumors in mouse lungs/ mice injected
v-SCLC					
N417	2×10^6	4	8/9 (89%)	17/27 (63%)	0/9
N417T1*	1×10^6	5	4/4 (100%)	7/8 (88%)	0/4
H82	2×10^6	4	8/10 (80%)	22/30 (73%)	0/10
H82T1*	1×10^6	5	4/5 (80%)	7/10 (70%)	0/5
H446	$1–3 \times 10^6$	15	9/15 (60%)	12/30 (40%)	0/15
H446T1*	$1–2 \times 10^6$	8	12/15 (80%)	19/30 (63%)	0/15
c-SCLC					
ACC-LC-51	$2–4 \times 10^6$	12–20	0/4 (0%)	0/12 (0%)	0/4
ACC-LC-60	2×10^6	12–20	0/8 (0%)	0/24 (0%)	0/8
ACC-LC-52	$2–4 \times 10^6$	12–20	1/20 (5%)	1/60 (2%)	0/20
ACC-LC-52T1*	2.5×10^6	16	2/20 (10%)	2/40 (5%)	0/20
H345	2×10^6	12	3/5 (60%)	4/10 (40%)	0/5
H146	$1–2 \times 10^6$	11	5/15 (33%)	5/30 (17%)	0/15
H146T1*	1.5×10^6	11	6/9 (67%)	7/18 (39%)	0/9
H69	2×10^6	9–10	2/5 (40%)	2/10 (20%)	0/5

The metastatic behavior of v-SCLC versus c-SCLC cell lines was significantly different in this metastasis assay ($P = 0.04$).
*Cell lines established from HFL tumors.

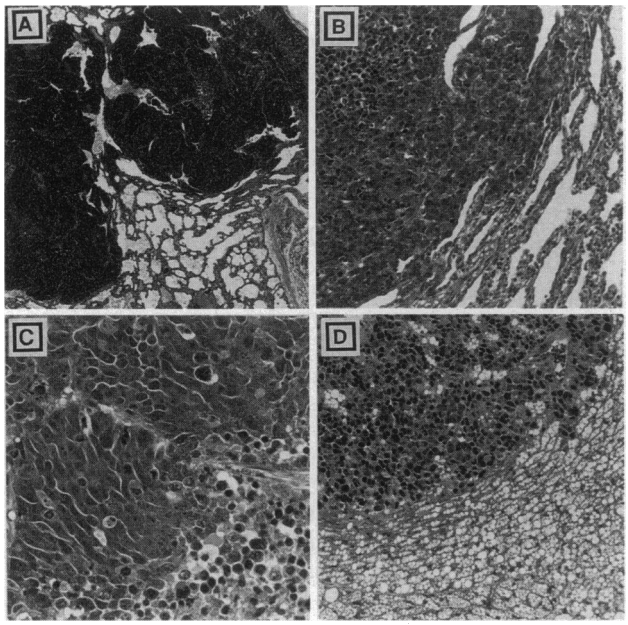

FIG. 2. SCLC tumors in SCID-hu-L. (*A*) Tumor nodules of N417 origin in HFL implant (×20). (*B*) Tumor of N417 origin in HFL implant bordered by remaining normal lung tissue (×110). (*C*) Tumor of H82 origin in HFL implant showing area of necrosis (×215). (*D*) Tumor of N417 origin in mouse brown fat tissue (×110).

itive for neuron-specific enolase, retaining the phenotypic profile of the original cell line (13, 16, 17).

Only three of six c-SCLC cell lines tested metastasized to HFL tissues with significant frequency (Table 1) and with longer latent periods of tumor growth than v-SCLC lines N417 and H82 (Table 1). Selection for variants with higher metastatic ability from cell lines ACC-LC-52 and H146 resulted in only a moderate increase in the frequency of metastases.

A salient feature of experimental metastasis of SCLC in SCID-hu-L was its specificity toward the human lung tissue. Metastatic lesions were not found in mouse lungs of any of the experimental animals (Table 1). Six of the nine SCLC lines tested were not metastatic toward any mouse tissues. Cell lines N417 and H82 produced ovarian tumors in ≈30–50% of the experimental female SCID mice. Cell lines N417 or H69 frequently produced tumors in several anatomical sites, such as the interscapular region, adjacent to but not involving the kidneys, and along major blood vessels in the thorax and abdomen. Histologic examination revealed that all these metastatic tumors were localized in the murine brown fat tissues (Fig. 2*D*). However, the efficiency of metastases to mouse tissues was low and critically depended on the number of cells injected. Thus, as mentioned above, injection of 10^4 N417 cells resulted in development of the HFL tumors in SCID-hu-L, but none of these animals have developed tumors in mouse tissues (data not shown).

To exclude the possibility that metastasis of v-SCLC to HFL implants is a consequence of grafting procedure, N417 cells were injected into SCID mice engrafted with newborn mouse lungs. No tumors developed in mouse lung implants, confirming the species-specificity of SCLC metastasis in SCID-hu. As a control for the tissue-specificity of metastases of v-SCLC to HFL grafts, N417 cells were injected into SCID mice engrafted with fragments of human fetal intestine (Fig. 1*D*). Despite a high number of cells injected (3×10^6), none of the eight injected mice developed tumors in the intestinal grafts. We conclude that metastasis of SCLC cells to HFL is both species- and tissue-specific.

To test whether v-SCLC cells are capable of spontaneous metastasis to HFL in SCID-hu-L, 5×10^4 N417T1 cells were injected directly into one of the two HFL implants of five mice, resulting in localized tumor growth. Five to 7 weeks after injection, two of five mice developed metastases in the second, uninjected HFL implant. Surgical removal of the primary tumor in the injected HFL resulted in metastatic growth in the remaining second HFL implant in five of five mice, whereas the site of resection showed no signs of tumor recurrence (data not shown). H82 cells were only weakly metastatic in spontaneous metastasis assays, with the frequency of metastasis to second HFL not exceeding 20%. These experiments demonstrate the applicability of the SCID-hu-L model for studying spontaneous metastases.

Metastasis of v-SCLC to HFBM in SCID-hu-BM. Metastasis to bone marrow is a frequent clinical feature of SCLC tumors (18). We attempted to reproduce this characteristic of SCLC in an experimental metastasis model using SCID-hu-BM (15). Previous studies (15) showed that implantation of human bone fragments into SCID mice results in the temporary disappearance of hematopoietic cells followed by recovery at ≈6 weeks after implantation. Early (i.e., <6 weeks after implantation) HFBM grafts contain stromal and vascular elements as the major cell types within the HFBM medulla (Fig. 3*A*). HFBM grafts of >6 weeks after implantation ("late") demonstrate multilineage human hematopoiesis (ref. 8 and Fig. 3*B*). To encompass these changes, cells of v-SCLC lines H82 and N417 were injected i.v. into SCID-hu-BM at different times after implantation of HFBM. At 5 to 7 weeks after injection, the presence of tumor cells within HFBM grafts was analyzed by histology and by *in vitro* culture of bone marrow cells. When tumor growth occurred within HFBM grafts, it was usually macroscopically evident due to the osteolytic nature of the tumors (see below and Fig. 3*C*).

Experimental metastasis of v-SCLC to HFBM was found to critically depend on the postimplantation age of the grafts; a significantly higher number of tumors developed in early grafts (Table 2). Thus, H82 cells metastasized efficiently to early HFBM grafts but metastasized only sporadically to late grafts (Table 2). Cell line HB1, derived from the only tumor that has developed in late HFBM graft had retained the metastatic preference for early HFBM (Table 2).

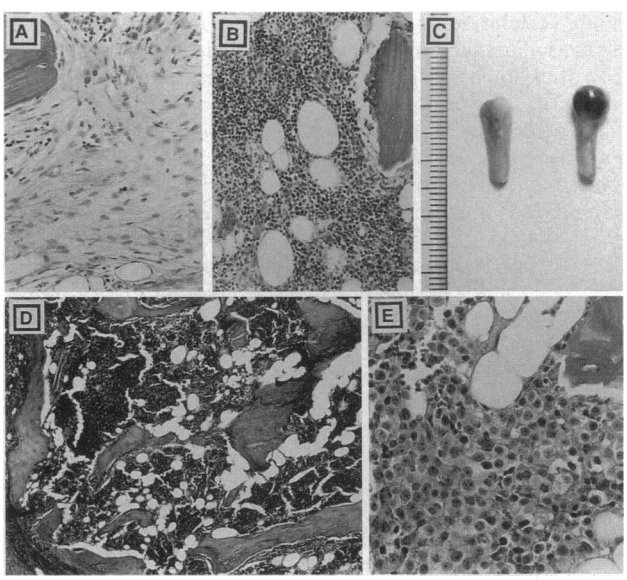

FIG. 3. SCLC tumors in HFBM implanted in SCID mice. (*A*) HFBM graft at 5.5 weeks after implantation (×100). (*B*) HFBM graft at 13 weeks after implantation (×100). (*C*) Macroscopic view of two HFBM grafts from SCID-hu-BM injected with N4BM4 cells. The bone on the right has developed a tumor. (*D* and *E*) Osteolytic tumors of H82 origin in HFBM graft (×40 and ×100, respectively).

Table 2. Metastasis of SCLC to HFBM of different postimplantation age

Injected cells*	HFBM age at injection, weeks	Mice with tumors in HFBM/mice injected	Mice with tumors in mouse bone marrow/ mice injected
H82	6–10	1/15 (7%)	ND
	3–6	6/11 (55%)	2/11 (18%)
HB1	6–10	2/15 (13%)	1/15 (7%)
	3–6	8/17 (47%)	4/17 (24%)
N417	6–10	1/10 (10%)	ND
	3–6	1/12 (8%)	1/12 (8%)
N4BM†	6–10	8/97 (8%)	11/97 (11%)
	3–6	24/37 (65%)	8/37 (22%)

The frequencies of metastases to early versus late HFBM grafts are significantly different ($P = 0.02$). ND, not determined.
*SCID-hu-BM were injected with 2–3 × 10^6 cells (H82 and N417) or 10^6 cells (HB1 or N4BM) per mouse; all mice were sacrificed at 5 to 7 weeks after injection.
†Combined data for 10 cell lines isolated in four consecutive rounds of selection.

v-SCLC N417 had a very low metastatic potential to HFBM of any postimplantation age (Table 2). A cell line (N4BM1) was re-established from a rare tumor induced by N417 in late HFBM grafts and was found to be highly metastatic to early, but not to late, HFBM grafts. Tumor cells were retrieved to establish cell lines that were, in turn, injected into SCID-hu-BM. This serial passage of bone marrow-metastatic variants of N417 (N4BM) in SCID-hu-BM has been continued through four rounds of i.v. injections, resulting in establishment of 10 metastatic variants; all were highly metastatic to early but not to late HFBM (Table 2). The tumors in HFBM were aggressive and inevitably led to the displacement of the normal bone marrow cellular elements and to osteolytic destruction (Fig. 3 *D* and *E*).

Both SCLC lines and their HFBM-metastatic variants were capable of infrequent metastases to mouse bone marrow. However, selection of variants of N417 metastatic to HFBM was not associated with a significant increase in frequency of metastases to mouse bone marrow (Table 2). Metastatic lesions in mouse vertebral bone marrow were usually limited to one vertebra and were small enough to remain confined to the medullar space. This result suggests that metastasis to and growth of human SCLC in the mouse bone marrow can occur but not as efficiently as in HFBM.

To exclude the possibility that metastasis of SCLC to HFBM is an artifact of engraftment, SCID mice were engrafted with bones from newborn SCID mice. The bone fragments implanted into mammary fat pads showed the presence of morphologically normal marrow within 4 weeks after implantation (data not shown). Cells of N4BM4, a cell line established after four rounds of selection *in vivo* for variants metastatic to HFBM, were injected into SCID mice engrafted with exogenous SCID bone marrow. None of the nine injected mice developed metastases to the SCID bone marrow grafts, even at 8 weeks after injection. This result further confirms the species-specific nature of bone marrow metastases of SCLC.

Effect of γ-Radiation and IL-1α on Metastasis of v-SCLC to Late HFBM. We addressed the possible reasons for the observed specificity of metastasis of v-SCLC to HFBM at early postimplantation times. We hypothesized that the abundant bone marrow stroma in early HFBM may actively produce cytokines and/or express specific adhesion molecules necessary to support the ensuing human hematopoiesis, thus creating favorable conditions for the homing and/or proliferation of metastatic cells. We tried to induce similar conditions in the late HFBM implants by subjecting SCID-hu-BM to sublethal doses of γ-irradiation. Sublethal irradiation of SCID-hu-BM results in massive death of hematopoietic cells in the HFBM followed by recovery (19), similar to the events observed in HFBM grafts after implantation (15).

SCID-hu-BM at 8–10 weeks after implantation were subjected to sublethal whole-body irradiation before injection of N4BM4 cells. Results in Table 3 show that irradiation at 1 or 3, but not 7 days, before injection results in metastasis of N4BM4 cells to late HFBM, whereas control unirradiated mice did not develop tumors in HFBM. Irradiation had no effect on metastasis to mouse bone marrow (Table 3) or to mouse brown fat (data not shown). Irradiation of SCID-hu-BM before injection of the parental N417 cells did not result in the increased frequency of metastasis to HFBM (Table 3). We conclude that sublethal irradiation activates a temporary response in late HFBM grafts that augments the species-specific metastasis of SCLC variants.

To ascertain that the effect of irradiation on metastasis to HFBM is not due to the damage and the loss of integrity of the endothelial barrier, experimental mice were treated with cyclophosphamide, an agent known to induce morphological changes in bone marrow endothelium similar to those induced by irradiation (20, 21). None of these mice has developed metastases in HFBM grafts upon subsequent injection with N4BM4 cells or the parental line N417 (Table 3). Thus, enhancement of metastasis to HFBM grafts is not likely to result from irradiation-induced permeability of bone marrow endothelium.

We examined next whether metastases of SCLC cells to late HFBM grafts could be induced through the treatment of SCID-hu-BM with IL-1α, a cytokine known to induce expression of specific adhesion molecules and growth factors by vascular endothelium and bone marrow stroma (22–25). If adhesion molecules and/or growth factors that are induced by IL-1α are involved in the metastasis of SCLC cells to HFBM grafts, enhancement of experimental metastasis to late HFBM in SCID-hu-BM might occur. Indeed, as shown in Table 3, a dramatic increase in species-specific metastasis to late HFBM was observed in SCID-hu-BM that were injected with IL-1α

Table 3. Effect of irradiation, cyclophosphamide, and IL-1α treatment on the metastasis of SCLC to late HFBM in SCID-hu-BM

Injected cells	Treatment prior to injection of SCLC cells	Time between treatment and injection of tumor cells	Mice with tumors in HFBM	Mice with tumors in mouse bone marrow
N4BM4	None		0/10	1/10
N4BM4	3 Gy	1 day	2/5	2/5
N4BM4	2 Gy	1 day	4/5	0/5
N4BM4	2 Gy	3 days	6/10	0/10
N4BM4	2 Gy	7 days	0/5	0/5
N417	2 Gy	1 day	0/9	0/9
N417	2 Gy	3 days	0/5	1/5
N4BM4	CY (100 mg/kg)	1 day	0/10	0/10
N417	CY (100 mg/kg)	1 day	0/4	0/5
N4BM4	IL-1α (100 ng) i.p.	3 hr	4/5	0/5
N4BM4	IL-1α (250 ng) i.p.	3 hr	3/5	0/5
N4BM4	IL-1α (100 ng) i.v.	1 hr	2/4	0/4
N417	IL-1α (100 ng) i.p.	1 hr	0/5	0/5
N417	IL-1α (100 ng) i.v.	1 hr	0/4	0/4
H82	IL-1α (100 ng) i.p.	3 hr	7/10	2/10
H82	2 Gy	1 day	1/10	3/10

All mice received i.v. injection of 10^6 cells indicated and were sacrificed at 5–6 weeks after injection. Two independent bone marrow metastatic variants of N417 isolated in the fourth round of selection were used. Whole-body irradiation (Gy) was from a ^{137}Cs source. CY, cyclophosphamide, injected i.p.

before injection of SCLC cells. As with radiation treatment, this effect was restricted to the selected metastatic variants N4BM4 and was not observed with the parental N417 cells.

IL-1α treatment, but not irradiation, had an inducing effect on metastasis of v-SCLC H82 to late HFBM (Table 3). This difference in metastatic behavior between H82 and N4BM4 cells could be associated with the different pattern of expression of adhesion molecules on these cell lines (unpublished results).

DISCUSSION

In this paper we have shown that the SCID-hu model can be used to study metastasis of human SCLC in a species- and tissue-specific manner. The major advantage of using SCID-hu mice in metastasis assay is that this model allows the metastatic spread of human tumor cells to human tissues. Thus, SCLC cells metastasized to HFL and HFBM tissues implanted into SCID mice, providing support for our hypothesis that some human tumor cells that are unable to lodge and grow in mouse tissues might be able to metastasize when presented with a human tissue microenvironment in SCID-hu mice.

Experimental and spontaneous metastasis of SCLC cells to HFL tissues was exquisitely species-specific, which is remarkable considering the following facts: (*i*) mouse lungs encompass the first major capillary bed encountered by the i.v. introduced cells, and (*ii*) mouse lungs are vastly superior in size and vascularization when compared with the HFL implants. The tissue-specificity of metastases in SCID-hu mice was demonstrated by the lack of metastases of SCLC to human intestinal grafts. Further evidence of tissue specificity of metastases was obtained from our unpublished experiments with neuroblastoma cell lines, showing efficient metastases of neuroblastoma cell lines to HFBM, but not HFL grafts in SCID-hu mice, in accordance with the clinical findings.

Our results on experimental metastasis of SCLC in SCID-hu-L demonstrate the differences in metastatic behavior of v- versus c-SCLC and reflect the existing clinical differences between the two subtypes (26). v-SCLC metastasis to HFL tissues in SCID-hu-L is more efficient than that of c-SCLC. The slower growth of tumors of c-SCLC origin in HFL tissue might reflect the generally lower proliferation rate of c-SCLC than v-SCLC cells *in vitro* (13). We did not observe a direct correlation between the metastatic potential and the degree of amplification or overexpression of the family of *myc* protooncogenes in SCLC lines.

SCID-hu-BM proved a valuable model for the study of hematogenous spread of SCLC cells to bone marrow. Preferential metastasis of SCLC to HFBM rather than mouse bone marrow is indicative of the role that species-specific interactions play in the metastasis of SCLC. In addition, neither whole-body irradiation nor systemic treatment with IL-1α (that is biologically active on murine and human cells) enhanced metastasis of SCLC to mouse bone marrow.

Experimental metastasis of v-SCLC cells to HFBM occurs with significant frequency only to HFBM grafts of early postimplantation age. Any or all of the following considerations might be important in understanding this preference. (*i*) It is possible that the human endothelial vasculature present in the early HFBM grafts is replaced by the murine endothelium at the later postimplantation times. However, our preliminary results indicate a long-term persistence of human endothelium in human grafts. (*ii*) The presence of mature human hematopoiesis in late grafts and specifically cellular elements of nonspecific immune response, such as natural killer cells, might interfere with the development of metastases. (*iii*) The bone marrow stroma in the early HFBM grafts might be particularly active in production of growth factors and adhesion receptors necessary to support the following dramatic recovery of the hematopoietic compartment of the grafts. Our results on enhancement of metastases to late HFBM by IL-1α or irradiation treatment appear to be compatible with the last possibility.

Our preliminary results indicate that species-specific metastases in SCID-hu mice can also be observed with human cell lines derived from solid tumors other than SCLC. The availability of this small animal model should enable the identification and analysis of important features of the human metastatic process that could not be addressed in conventional metastasis models.

The essential contribution of the Systemix Animal Production and Transplant Group to this work is gratefully acknowledged. We are indebted to Dr. J. M. McCune for his continuous support, encouragement, and critical review of the manuscript; to Dr. H. Kaneshima for valuable discussions; and to Y. Shtivelman for statistical analysis of the data. We thank Dr. T. Takahashi (Aichi Cancer Center, Nagoya) for the gift of cell lines and Drs. L. Su and M. Bonyhadi for the careful reading of the manuscript. Irving L. Weissman is a co-founder of and consultant to Systemix Inc.

1. Fidler, I. J. (1986) *Cancer Metastasis Rev.* **5,** 29–49.
2. Sharkey, F. E. & Fogh, J. (1984) *Cancer Metastasis Rev.* **3,** 341–360.
3. Giavazzi, R., Campbell, D. E., Jessup, J. M., Clearly, K. & Fidler, I. J. (1986) *Cancer Res.* **46,** 1928–1933.
4. McLemor, T. L., Liu, M. C., Blacker, P. C., Gregg, M., Alley, M. C., Abbott, B. J., Shoemaker, R. H., Bohlman, M. E., Litterst, C. C., Hubbard, W. C., Brennan, R. H., McMahon, J. B., Fine, D. L., Eggleston, J. C., Mayo, J. G. & Boyd, M. R. (1987) *Cancer Res.* **47,** 5132–5140.
5. Morikawa, K., Walker, S. M., Nakajima, M., Pathak, S., Jessup, J. M. & Fidler, I. J. (1988) *Cancer Res.* **48,** 6863–6871.
6. Yamashita, T. (1989) *J. Jpn. Soc. Cancer Ther.* **24,** 611–616.
7. Waller, E. K., Kamel, O. W., Cleary, M. L., Majumdar, A. S., Schick, M. R., Lieberman, M. & Weissman, I. L. (1991) *Blood* **78,** 2650–2665.
8. Namikawa, R., Ueda, R. & Kyoizumi, S. (1993) *Blood* **82,** 2526–2536.
9. Juhasz, I., Albelda, S. M., Elder, D. E., Murphy, G. F., Adachi, K., Herlyn, D., Valyi-Nagy, I. T. & Herlyn, M. (1993) *Am. J. Pathol.* **143,** 528–537.
10. Bosma, G. C., Custer, R. P. & Bosma, M. J. (1983) *Nature (London)* **301,** 527–530.
11. McCune, J. M., Namikawa, R., Kaneshima, H., Shultz, L. D., Lieberman, M. & Weissman, I. L. (1988) *Science* **241,** 1632–1639.
12. Carney, D. N. & Minna, J. D. (1982) *Clin. Chest Med.* **3,** 389–398.
13. Carney, D. N., Gazdar, A. F., Bepler, G., Guccion, J. G., Marangos, P. J., Moody, T. W., Zweig, M. H. & Minna, J. D. (1985) *Cancer Res.* **45,** 2913–2923.
14. Takahashi, T., Obata, Y., Sekido, Y., Hida, T., Ueda, R., Watanabe, H., Ariyoshi, Y., Sugiura, T. & Takahashi, T. (1989) *Cancer Res.* **49,** 2683–2688.
15. Kyoizumi, S., Baum, C. M., Kaneshima, H., McCune, J. M., Yee, E. J. & Namikawa, R. (1992) *Blood* **79,** 1704–1711.
16. Bunn, P. A., Linnoila, I., Minna, J. D., Carney, D. & Gazdar, A. F. (1985) *Blood* **65,** 764–768.
17. Jensen, S. M., Gazdar, A. F., Cuttitta, F., Russell, E. K. & Linnoila, R. I. (1990) *Cancer Res.* **50,** 6068–6074.
18. Abrams, J., Doyle, L. A. & Aisner, J. (1988) *Semin. Oncol.* **15,** 261–277.
19. Kyoizumi, S., McCune, J. M. & Namikawa, R. (1994) *Radiat. Res.* **137,** 76–83.
20. Shirota, T. & Tavassoli, M. (1991) *Exp. Hematol.* **19,** 369–373.
21. Shirota, T. & Tavassoli, M. (1992) *Blood Cells* **18,** 197–214.
22. Bevilacqua, M. P., Pober, J. S., Wheller, M. E., Cotran, R. S. & Gimbrone, M. A., Jr. (1985) *J. Clin. Invest.* **76,** 2003–2011.
23. Zukali, J. R., Dinarello, C. A., Oblon, D. J., Gross, M. A., Anderson, L. & Weiner, R. S. (1986) *J. Clin. Invest.* **77,** 1857–1863.
24. Bagby, G. C., Jr., Dinarello, C. A., Wallace, P., Wagner, C., Hefeneider, S. & McCall, E. (1986) *J. Clin. Invest.* **78,** 1316–1322.
25. Fibbe, W. E., van Damme, J., Billiau, A., Goselink, H. M., Voogt, P. J., van Eeden, G., Ralph, P., Altrock, B. W. & Falkenburg, J. H. (1988) *Blood* **71,** 430–435.
26. Cook, R. M., Miller, Y. E. & Bunn, P. A., Jr. (1993) *Curr. Probl. Cancer* **17,** 69–141.