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SCID-hu mice for the study of human cancer metastasis

Abstract Cancer metastasis involves dynamic and multistep in vivo processes. While generation of metastatic clones requires genetic alterations in cancer cells, subsequent selection of the clones is heavily influenced by interactions with the surrounding tissue microenvironment. To reproduce the complex cellular interactions that occur in human patients is, however, difficult, and has not been achieved using currently available in vitro systems or conventional animal models. The SCID-hu mouse is generated by surgical implantation of human fetal tissues into mutant mice of the severe combined immunodeficient (SCID) phenotype. The unique feature of this model is that the implanted human tissues maintain their normal architecture and function. Therefore implanted human tissues will provide relevant microenvironments for the growth and metastasis of human cancer cells. The SCID-hu mouse model, which was specifically designed for the study of human cancer biology, enables experimental investigation of cellular events involved in cancer metastasis on the basis of interactions between human cancer cells and the human tissue microenvironment. It has been demonstrated that various types of human cancer cell lines generate tumors in implanted human bone marrow and lung, organs frequently involved in metastasis in patients, upon intravenous inoculation. Tumorigenic activity in SCID-hu mice faithfully reflects the clinical features of the original cancer. Tumor formation and selection of high tumorigenic variants occur in a species-specific manner. Furthermore, it was shown that metastatic tumor formation is regulated by both cancer cells and conditions in the host organs. Conditioning of animals by either whole-body irradiation or interleukin 1α treatment prior to cancer cell inoculation induced metastatic tumor formation by certain small cell lung cancer (SCLC) cell lines specifically in human bone marrow. A novel gene has been identified by comparing gene expression profiles between high and low tumorigenic SCLC cells in human lung. This gene is preferentially expressed in low metastatic lines, and transfection of the gene into highly metastatic cells results in suppression of metastasis. Recent studies have shown that the gene product is involved in the apoptosis induction pathway. Collectively, our results indicate that the SCID-hu mouse will serve as a unique platform technology with which to investigate cellular events involved in human cancer metastasis, as well as to identify genes playing important roles in the growth and metastasis of human cancer, in the context of interactions between human cancer cells and human tissue environments.

Key words Metastasis · Animal model · SCID-hu mouse

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Introduction

Despite significant advances in our understanding of cancer biology and cancer treatments in the past decades, cancer is still the second leading cause of death in the USA. The cause of treatment failure in advanced cancer is mainly the generation of cancer clones with the ability to metastasize to distant organs. However, little is known about the molecular basis of metastatic processes. Metastases involve highly selective, multistep in vivo events. Selection occurs at each step of metastasis on the basis of interactions between cancer cells and the surrounding tissue environment.

In a concept reintroduced by Fidler after a century [2], in 1889 Paget stated that the process of metastasis is

not due to chance but that certain tumor cells (the "seed") have a specific affinity for the milieu of certain organs (the "soil") based on careful examination of autopsy records of breast cancer patients [14]. Metastases will result only if the "seed" and "soil" are matched because cancer cells, although their growth is somewhat dysregulated, can proliferate only when they are adequately supported by surrounding host tissues. Fidler, based on decades of pioneering work underscoring the roles of tissue environments in cancer biology, concluded that the ideal in vivo model for studying human cancer should allow the interaction of tumor cells with their relevant organ environment [2]. To reproduce the complicated interactions that occur in human patients is, however, difficult, and has not been achieved using currently available in vitro systems or conventional animal models.

SCID-hu mice are generated by surgical transplantation of human fetal organs into mutant mice of the severe combined immunodeficient (SCID) phenotype [4, 8, 12]. The unique feature of this model is that the human cells proliferate, differentiate, and function in implanted human tissues that maintain their normal anatomic architecture. We have demonstrated that this animal model provides a relevant tool to study the development and function of human hematolymphoid organs [3, 15], the pathogenesis of a broad range of infectious diseases [1, 10, 11], and the efficacy of various therapeutic agents [5, 6, 9]. We review here the models specifically designed to investigate the mechanisms involved in metastasis of human cancer cells in human tissues [16, 17]. Human lung and bone marrow (BM), organs which are frequently involved in metastasis in patients, were used as host organs for the metastasis. Our results demonstrate that the SCID-hu mouse, which enables experimental investigation of human cancer metastasis with the most relevant combination of seed and soil, provides a useful tool to understand the molecular mechanisms underlying the metastatic processes of human cancer cells.

Methods

Homozygous 6–8-week-old C.B-17 *scid*/*scid* mice were used. Two or more small pieces of human fetal lung at 18–22 weeks gestational age were surgically transplanted into the fourth mammary fat pads of mice under anesthesia (SCID-hu-FL) [17]. Similarly, a fragment of human fetal long bone containing BM was subcutaneously transplanted (SCID-hu-BM) [4]. Mice were used for experiments 7–8 weeks after transplantation, unless otherwise stated.

The human cancer cell lines used in the experiments were obtained from the American Type Culture Collection (ATCC), unless stated specifically. These cell lines were subjected to an in vivo metastasis assay. In brief, cultured cells were harvested by trypsinization or centrifugation and resuspended in Hanks' balanced saline solution (HBSS); $1–3 \times 10^6$ cells were injected into SCID-hu mice intravenously through the lateral tail vein. Tumor formation was assessed macroscopically and confirmed by histological examination. In some experiments, cancer cells were retrieved from the tumor generated in human tissues, expanded in vitro, and subjected to another in vivo metastasis assay.

Results and discussion

Human tissues in the SCID-hu mouse support the growth of human cancer cells efficiently

Thirty cell lines established from a variety of human cancers including lung cancer (adenocarcinoma and small cell lung cancer [SCLC]), breast cancer, prostate cancer, colon cancer, neuroblastoma, and myeloid leukemia) were subjected to an in vivo metastasis assay using SCID-hu-FL mice. Of those, 16 (53%) generated tumors in human lung grafts after intravenous injection. Similarly, 10 of 26 cell lines (38%) generated tumors in human BM grafts. It is interesting to note that cell lines derived from SCLC, prostate cancer, and neuroblastoma generated tumors in human BM at high frequency, reflecting the clinical features of these cancer cells which preferentially metastasize to BM in patients. It was also found for some cell lines that injection of as few as 10^4 cells induced metastatic tumor formation in human tissues.

To exclude the possibility that metastases produced by human cancer cells are the consequence of the grafting procedure, cells were injected into SCID mice engrafted with newborn mouse lung or bone in a manner similar to that used to create SCID-hu mice. No tumors developed in the grafts in these control experiments, confirming the species specificity of human cancer cell metastases in SCID-hu mice.

In another experiment [13], it was demonstrated that various types of leukemia cells obtained from patients at the time of diagnosis generate tumors in human BM after direct injection into BM grafts. Leukemia cells, especially of the myeloid phenotype, have been known to be difficult to propagate in animals. Successful implantation of various leukemia cells in the human BM microenvironment underscores its importance in supporting the growth of human leukemia cells.

These results together demonstrate that implanted human tissues provide appropriate environments to support the efficient growth of various human cancer cells.

Growth of human cancer cells in SCID-hu mice reflects clinical features

As described above, cell lines derived from SCLC, prostate cancer, and neuroblastoma show high metastatic activity for human BM, reflecting the high affinity of these cells for BM tissue observed in patients. In addition, results that further support the clinical relevance of the model were obtained. First, the tumorigenic activity of cell lines derived either from the more aggressive and refractory variant SCLC (v-SCLC) or from the classic SCLC (c-SCLC) were investigated using SCID-hu-FL mice [17]. As summarized in Table 1, cell lines derived from v-SCLC generated tumors in human

Table 1 Growth of human cancer cells in SCID-hu mice reflects clinical features

Injected cell line	Latency (weeks)	% of animals with metastasis to	
		Human lung	Mouse lung
SCLC cell lines			
c-SCLC			
ACC-LC-51[a]	12–20	0	0
ACC-LC-60[a]	12–20	0	0
ACC-LC-52[a]	12–20	5	0
H345	12	60	0
H146	11	33	0
H69	9–10	40	0
v-SCLC			
N417	4	89	0
H82	4	80	0
H446	15	60	0
Colon cancer cell lines			
Primary			
SW480	14–15	0	86
T34[b]	14–15	0	0
Metastatic			
SW620	8–10	40	40
COLO320DM	8–10	100	0

[a] Cell lines provided by T. Takahashi, Aichi Cancer Center, Nagoya, Japan

[b] Cell line established in our laboratory

lung grafts much more frequently and with shorter latency than c-SCLC cell lines. None of the lines tested generated tumors in the mouse lung. Second, colon cancer cell lines derived either from primary lesions of patients with no sign of metastasis or from metastatic lesions were compared for tumorigenic activity in SCID-hu-FL mice [16]. SW480 and T34, cell lines established from primary lesions, did not generate tumors in human lung after up to 15 weeks, while SW620 and COLO320DM, derived from metastatic lesions, generated tumors 8 to 10 weeks after intravenous injection. SW620 was established from a metastatic lymph node of the same patient whose primary tumor was used to establish SW480 [7]. Both SW480 and SW620 generated metastatic nodules in mouse lung, with a higher frequency of metastases from primary SW480 cells than metastatic SW620 cells. This contrasts with the metastatic activity observed in the patient as well as in human lung in SCID-hu mice.

In summary, it was shown that in SCID-hu mice 1) the affinity of certain cancer cells for specific organs observed in patients was reproduced; 2) cell lines derived from clinically more aggressive subtypes of cancer had higher tumorigenic activity; and 3) cell lines derived from metastatic lesions had higher tumorigenic activity than those derived from primary lesions. Thus the growth of human cancer cell lines in human tissues in SCID-hu mice faithfully reflects the clinical features of the original cancer.

Growth and selection of human cancer cells occur in a species-specific manner

We isolated high tumorigenic variants from low tumorigenic parental cells. In brief, cells were retrieved from a tumor that was generated by intravenous injection of low tumorigenic parental cells, expanded in vitro, and reinjected into another set of SCID-hu mice. By repeated in vivo passaging, variant cell lines that generate metastatic tumors in human organs more frequently than the parental cells were isolated (Table 2). The v-SCLC cell line N417 produced metastatic tumors in human BM in $<10\%$ of animals, while an in vivo-selected line (N4BM) derived from a N417 tumor metastasized in 65% of SCID-hu mice when injected 3 to 6 weeks after implantation of BM grafts [17] (see below). However, the metastatic activity of N4BM cells in mouse BM did not increase significantly, indicating that the in vivo selection of high metastatic variants occurred based on the species-specific interaction of N4BM cells and the human BM environment.

Similarly, high metastatic variants were isolated from the colon cancer cell line SW620 by passaging in SCID-hu-FL mice [16]. The percentage of lung grafts with tumors increased from 25% to 55% by the first

Table 2 Species-specific selection of high metastatic variants from human cancer cells (*BM* bone marrow)

Injected cell line	Latency (weeks)	% of animals with metastasis to	
		Human BM	Mouse BM
v-SCLC			
N417	5–7	8	8
N4BM	5–7	65	22
		Human lung	Mouse lung
Colon cancer			
SW620	8–10	40	40
SL1	8–10	90	20
SL2	8–10	90	0

passage (SL1) and to 65% by the second passage (SL2). Both SL1 and SL2 cells generated tumors in human lung in 90% of animals injected. SW620 cells generated a few metastatic nodules in the mouse lung (mean, 3.5 nodules per lung) in 40% of animals. However, after in vivo passage the frequency of metastasis in the mouse lung decreased, in contrast with the increases in metastatic frequency in human lung grafts. SL1 cells generated a mean of 0.3 metastatic nodules in 20% of animals, and SL2 cells completely lost the ability to generate metastatic nodules in mouse lung. Thus metastatic activities for human lung and in mouse lung were clearly dissociated in the SL2 cells. This implies that mechanisms underlying the metastasis of SW620 cells into mouse lung are different from those involved in the metastasis of SL2 cells into human lung. Together, these results illustrate the critical influence of species-specific tissue environments on selective processes in metastasis. Equivalent murine organs fail to reproduce the influences provided by relevant human organ environments.

Metastasis occurs only when the seed and soil are matched

During the analysis of metastatic activity of v-SCLC cell lines to human BM in the SCID-hu mouse, it was found that some cell lines metastasize frequently only in mice that are transplanted with fetal BM 3–6 weeks prior to injection (early grafts) [17]. These cell lines generate tumors at a low frequency when injected into mice with BM grafts transplanted 6–10 weeks before injection (late grafts). The cell line H82 metastasized in 55% of mice with early grafts, while only 7% of mice with late grafts developed tumors in human BM. Similarly, the variant N4BM, isolated from N417 cells, metastasized in 65% and 8% of mice with early and late grafts, respectively. The parental line N417 metastasized in $<10\%$ of mice regardless of the time after implantation.

Previous studies have shown that implantation of human BM into SCID mice results in the temporary disappearance of hematopoietic cells followed by recovery by 6 weeks after implantation [4]. Stable hematopoiesis is maintained thereafter until ≥20 weeks posttransplantation. Therefore we hypothesized that the abundant BM stroma in early grafts may actively produce cytokines and/or express specific adhesion molecules necessary to support the recovery of human hematopoiesis, thus creating favorable conditions for the homing and/or proliferation of SCLC cells. To test this hypothesis, we tried to induce similar conditions in the implanted human BM in a stable phase (7–10 weeks posttransplantation) by subjecting animals to sublethal whole-body γ-irradiation. Sublethal irradiation of SCID-hu-BM mice results in death of hematopoietic cells in the graft followed by recovery [6], similar to the events observed in BM grafts after implantation. As shown in Table 3, irradiation at 1 or 3 days, but not 7 days, before injection induced BM metastasis by N4BM cells in 60–80% of grafts, whereas metastasis by parental N417 cells was not affected by irradiation [17].

We next examined whether higher rates of metastasis could be induced by treating mice with recombinant human interleukin (IL) 1α, a cytokine known to induce the expression of specific adhesion molecules and growth factors in vascular endothelial cells and BM stromal cells. A significant increase in human BM metastasis by N4BM cells was induced in SCID-hu-BM mice pretreated with IL-1α (Table 3). Similar experiments were conducted with H82 cells, which frequently metastasize only in early BM grafts. IL-1α treatment, but not irradiation, showed an inducing effect on BM metastasis by H82 cells (Table 3). In none of these experiments were increases in metastasis to murine organs, including BM, observed.

These results provide evidence that supports the seed and soil hypothesis: certain tumor cells (the "seed") have a specific affinity for the milieu of certain organs (the "soil"). N4BM cells have an affinity for BM grafts

Table 3 Effects of BM conditioning on metastasis by SCLC cell lines (*NA* not applicable, *ND* not determined, *rhIL-1α* recombinant human interleukin 1α)

Injected cells	Conditioning	Time after treatment	% of animals with metastasis to	
			Human BM	Mouse BM
N417	None	NA	10	8
	γ-irradiation 2 Gy	1 day	0	0
		3 days	0	20
	rhIL-1α 100 ng ip	3 h	0	0
	rhIl-1α 100 ng iv	1 h	0	0
N4BM	None	NA	0	10
	γ-irradiation 2 Gy	1 day	80	0
		3 days	60	0
		7 days	0	0
	rhIL-1α 100 ng ip	3 h	80	0
	rhIl-1α 100 ng iv	1 h	50	0
H82	None	NA	7	ND
	γ-irradiation 2 Gy	1 day	10	30
	rhIL-1α 100 ng ip	3h	70	20

preconditioned either by irradiation or IL-1α treatment, while parental N417 cells have no affinity for BM regardless of the conditions and the affinity of H82 cells is restricted to BM conditioned by IL-1α treatment. Thus metastases are bidirectionally regulated, ie, by the features of cancer cells and by the host organ environment. It should be emphasized that these results indicate that the "soil" does not simply mean the tissue environment, but a particular condition(s) of the tissue environment. The fact that irradiated BM provides a milieu for N4BM cells but not for H82 cells indicates that the molecular bases for the metastases of these cell types are, at least in part, different, suggesting that the selective processes of metastasis are regulated by complex molecular mechanisms. Finally, neither whole-body irradiation nor IL-1α, a cytokine cross-reactive between human and mouse cells, induced metastasis of SCLC cells to murine organs, again indicating that the metastasis of N4BM cells and H82 cells is regulated by interactions between these cells and species-specific human BM environments.

Identification of molecules that play key roles in tumorigenic activities

A novel gene, CC3, that plays a critical role in determining the tumorigenic activity of SCLC cells was identified by comparing the gene expression profiles of high metastatic v-SCLC cell lines and low metastatic c-SCLC cell lines [18]. This gene is abundantly expressed in c-SCLC cell lines, while its expression is suppressed in v-SCLC cell lines. The CC3 gene does not have any homology to known or function-assigned sequences, yet homologous sequences are evolutionarily conserved.

Transfection of the CC3 gene into v-SCLC cells converts their biological behavior; cells that were highly metastatic to human lung in the SCID-hu mouse became low metastatic. Thus it was shown that lack of CC3 expression is the cause of the high metastatic activity of v-SCLC cells, not the effect of high metastatic activity induced by other mechanisms nor a simple bystander. Recent studies have demonstrated that the CC3 gene product is involved in apoptosis induction. Therefore lack of CC3 expression in v-SCLC cells may, at least in part, explain the higher resistance of v-SCLC to chemo- and radiotherapy. Furthermore, this observation provides evidence supporting a direct link between apoptosis and metastasis, underscoring the importance of apoptosis in cancer biology.

Conclusions

In conclusion, we have demonstrated that 1) human tissues in the SCID-hu mouse cancer model support the growth of various human cancer cells; 2) growth of human cancer cells in this model faithfully reflects clinical features; 3) growth and selection of human cancer cells are supported in a species-specific manner; 4) metastases of human cancer cells are bidirectionally regulated by cancer cells and the surrounding host organ environment; and 5) this model provides a unique tool to identify molecules that play key roles in determining tumorigenic activity. Identification of molecules directly involved in human cancer metastasis will provide appropriate targets for the discovery and development of cancer therapeutics with new modes of action.

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