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A Novel SCID Mouse Model for Studying Spontaneous Metastasis of Human Lung Cancer to Human Tissue

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We established a novel severe combined immunodeficient (SCID) mouse model for the study of human lung cancer metastasis to human lung. Implantation of both human fetal and adult lung tissue into mammary fat pads of SCID mice showed a 100% rate of engraftment, but only fetal lung implants revealed normal morphology of human lung tissue. Using these chimeric mice, we analyzed human lung cancer metastasis to both mouse and human lungs by subcutaneous inoculation of human squamous cell carcinoma and adenocarcinoma cell lines into the mice. In 60 to 70% of SCID mice injected with human-lung squamous-cell carcinoma, RERF-LC-AI, cancer cells were found to have metastasized to both mouse lungs and human fetal lung implants but not to human adult lung implants 80 days after cancer inoculation. Furthermore, human-lung adenocarcinoma cells, RERF-LC-KJ, metastasized to the human lung implants within 90 days in about 40% of SCID mice, whereas there were no metastases to the lungs of the mice. These results demonstrate the potential of this model for the *in vivo* study of human lung cancer metastasis.

Key words: SCID — Lung cancer — Metastasis

Models based on athymic nude mice have been used for human cancer research. Many human epithelial and hematopoietic tumors that do not grow in nude mice can grow in severe combined immunodeficient (SCID) mice[1] and induce diseases very similar to what is observed in humans.[2] Moreover, using human lung cancer and melanoma, SCID mice were found to be superior to nude mice in that metastatic frequency is higher and the time needed to metastasize is shorter in the former than in the latter.[3,4] We have also reported that the SCID mouse is an excellent animal model system for implantation of human lung cancer tissue dissected from patient specimens, that human adult normal lung tissue engraftment in SCID mice showed a 100% take rate, and that human lung squamous and adenocarcinoma cells metastasized more frequently in SCID mice than in nude mice.[5] Shtivelman and Namikawa have reported that human small cell carcinoma can metastasize to human lung and bone marrow implanted in SCID mice.[6] Here we describe a SCID mouse model which enables examination of metastasis of human squamous cell carcinoma and adenocarcinoma to human normal tissue by implanting both human tumor and normal tissue into the same recipient mouse.

The 6- to 10-week-old SCID mice were obtained from CLEA Japan, Inc. (Tokyo). Throughout the experiment, female mice were used and maintained under specific pathogen-free conditions. Fresh fragments of human fetal and adult normal peripheral lung tissue were obtained from patients at the time of surgery or pathological autopsy. The human-lung squamous-cell carcinoma cell line, RERF-LC-AI (RCB 0444, RIKEN Cell Bank, Tsukuba), was derived from pleural effusion at the time of pleural puncture.[7] Human-lung adenocarcinoma cell line, RERF-LC-KJ (in the process of registration at RIKEN Cell Bank), was established from cancer tissue grown in a SCID mouse after implantation of an autopsy specimen.[5] Both lines were grown in RPMI 1640 tissue-culture medium and supplemented with 10% fetal bovine serum, 100 units/ml penicillin, 100 μg/ml streptomycin, and 50 m*M* L-glutamine (complete RPMI 1640).

The peripheral lung tissues were finely minced into fragments of approximately 8 mm^3 and implanted into the mammary fat pads of SCID mice[8] anesthetized with ketamine and xylazine, as previously described. About one month after successful implantation of the lung tissues, one million RERF-LC-AI and RERF-LC-KJ in 50 μl of complete RPMI 1640 were subcutaneously implanted into the back of the mice. After 70–90 days, mice were killed, and their lungs, axillary lymphnodes, and human lung implants were examined histologically for metastases. Cancer nodules in mouse lung larger than 0.5 mm in diameter were histologically confirmed. All specimens were classified by routine histological examination.

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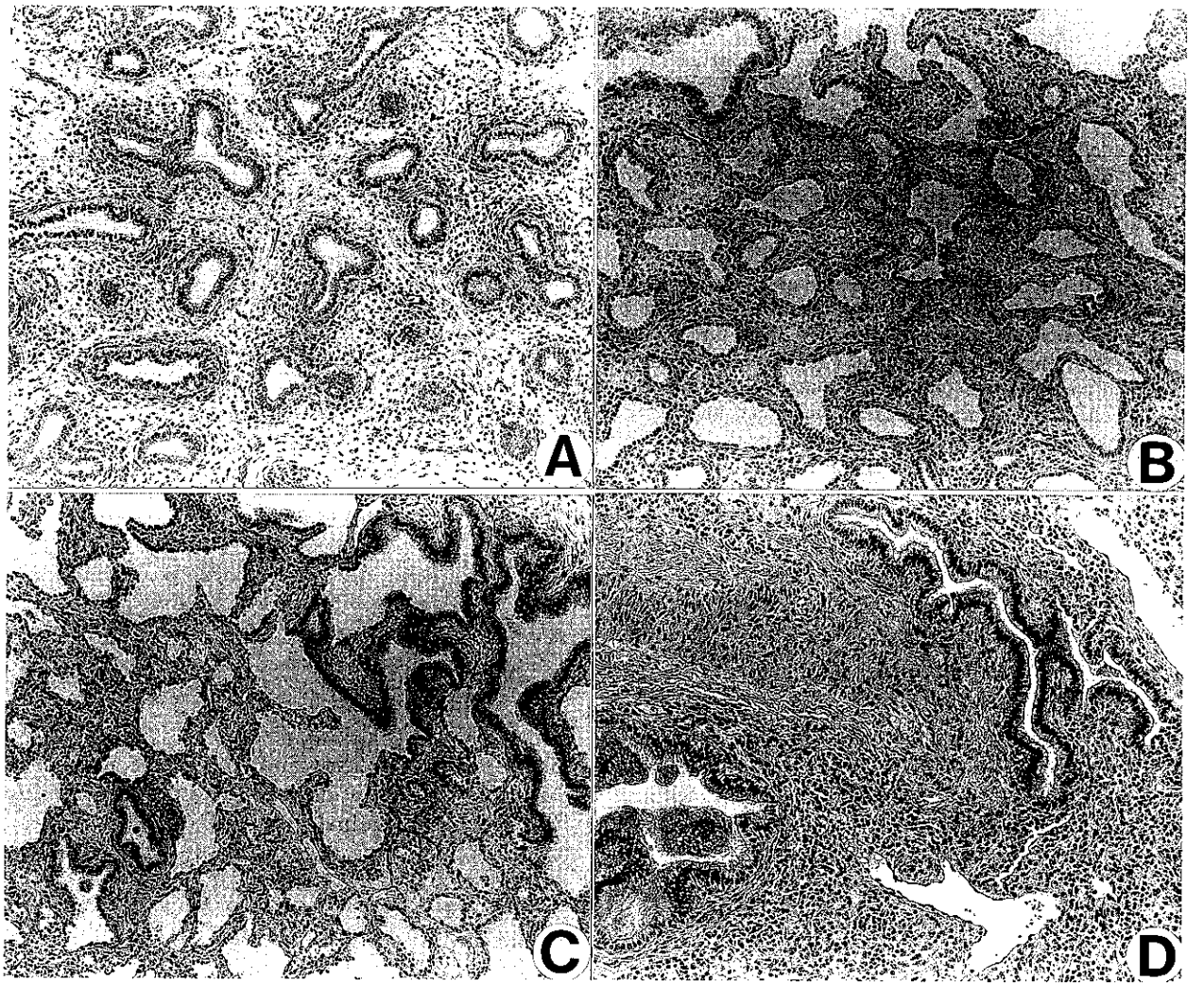

Fig. 1. Microscopic views of human fetal lung before implantation (A), 1 month after implantation into SCID mice (B), 3 months after implantation into SCID mice (C) and human adult lung 3 months after implantation into SCID mice (D) (Hematoxylin and eosin stain; magnification: ×30).

Table I. Frequency of the Metastasis of Implanted Human Lung Cancer to Implanted Human Fetal and Adult Normal Lung in SCID Mice

SCID mouse implanted with	Site of metastasis	RERF-LC-AI[b]	RERF-LC-KJ[c]
Human fetal lung[a]	Human fetal lung	5/8[d] (63%)	3/7 (43%)
	Mouse lung	6/8 (75%)	0/7 (0%)
	Mouse axillary lymphnode	2/8 (25%)	4/7 (57%)
Human adult lung[a]	Human adult lung	0/10 (0%)	ND[e]
	Mouse lung	10/10 (100%)	ND

a) The numbers of human donors of fetal normal lung and adult normal lung for the metastases of RERF-LC-AI are 3 and 2 cases, respectively and of human fetal normal lung for RERF-LC-KJ, 2 cases.
b) Human-lung squamous-cell carcinoma cell line.
c) Human lung adenocarcinoma cell line.
d) No. of mice showing metastasis/Total no. of mice.
e) Not done.

The origin of the metastases were confirmed by PCR (polymerase chain reaction)-SSCP (single-strand conformation polymorphism) analysis of HLA DQA1 gene.[9, 10] Tissues were held in 100 μl of digestion buffer (50 m*M* Tris-Cl, pH 8.5, 1 m*M* EDTA, 0.5% Tween 20) containing 100 μg of proteinase K at 56°C for 48 h. After phenol-chloroform treatment, genomic DNA was precipitated with ethanol. Subsequently genomic DNA (200 ng) was subjected to 35 cycles of PCR amplification of HLA classII DQA1* in 20 μl of solution containing 5 m*M* KCl, 10 m*M* Tris-HCl (pH 8.3), 1.5 m*M* $MgCl_2$, 200 μM each of four dNTPs, 2.5 pmol of PCR primers, and 0.5 unit of Taq DNA polymerase (Perkin-Elmer Cetus). The products of PCR were detected by 8% polyacrylamide gel electrophoresis followed by ethidium bromide staining. Forward and backward PCR primers for amplification of human HLA DQA1* were 5′-GTGCTGCAGGTGTAAACTTGTACCAG-3′ and 5′-CACGGATCCGGTAGCAGCGGTAGAGTTG-3′.

Human fetal peripheral lung tissues were implanted into ten SCID mice. In all mice, implanted tissues increased in size within one month. As shown in Fig. 1, A–C, development of alveolar and bronchial epidermal structures was observed about 3 months following implantation in all implants. Likewise, in the case of implantation of human adult peripheral lung tissue, typical

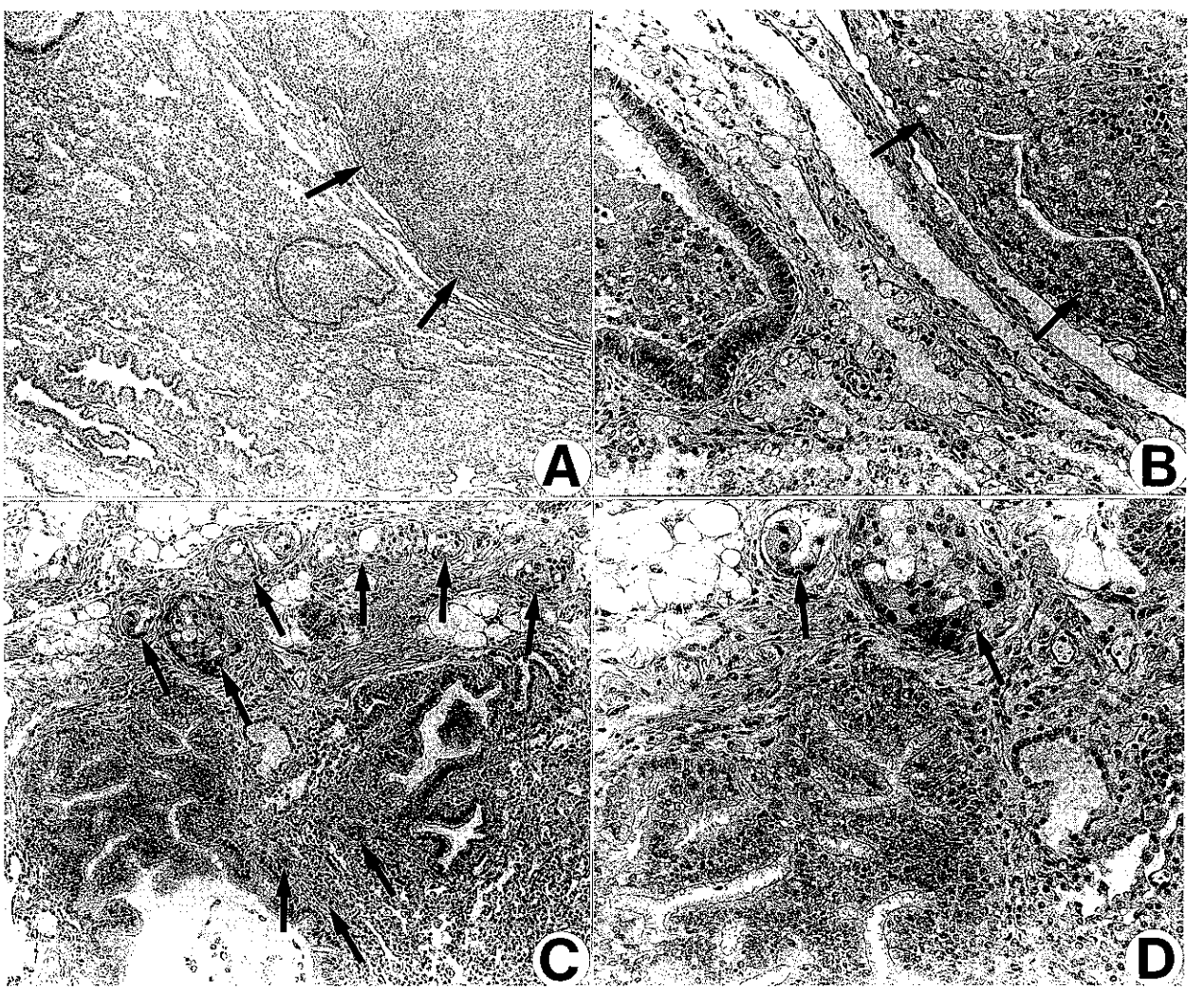

Fig. 2. Metastatic lesions of RERF-LC-AI (squamous-cell carcinoma) and RERF-LC-KJ (adenocarcinoma) in human fetal lung implanted in SCID mice. Upper columns (A and B) are RERF-LC-AI, and lower columns are RERF-LC-KJ. The arrows indicate the metastatic lesions surrounded by human alveolar tissue. [Hematoxylin and eosin stain; magnification: ×12 (A), ×30 (C), ×60 (B and D)].

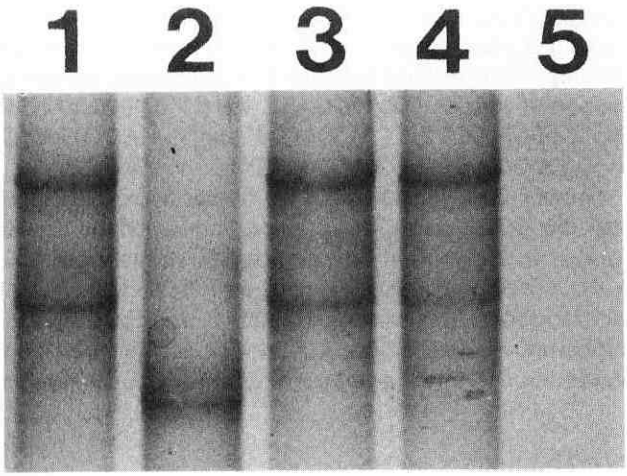

Fig. 3. HLA-DQA1* tissue typing using PCR-SSCP. Lane 1, RERF-LC-KJ; lane 2, human fetal lung; lane 3, lesion from human lung of lane 2 after implantation of RERF-LC-KJ; lane 4, same as lane 3; lane 5, SCID mouse tissue.

alveolar structures disappeared, but many bronchial epidermal features were observed in all implants (Fig. 1D). The growth of connective tissue was apparent within these bronchial structures. These results are consistent with our previous report.[5]

The frequency of metastasis of implanted human lung cancer to mouse lungs, mouse axillary lymphnodes and human lung implants in SCID mice is summarized in Table I. RERF-LC-AI metastasized to the human fetal lung implants in 5 of 8 SCID mice (63%) (Fig. 2, A and B), but there were no metastases to the human adult lung implants. Interestingly, RERF-LC-KJ metastasized to human fetal lung tissue in 3 of 7 SCID mice (43%) (Fig. 2, C and D), although there were no metastases to the mouse lungs. Furthermore, RERF-LC-AI metastasized frequently to mouse lungs, but less frequently to mouse lymphnodes. In contrast, RERF-LC-KJ metastasized only to lymphnodes, as previously observed.[5]

Fig. 3 shows representative results of PCR-SSCP analysis for confirming cancer origin. In the present study the HLA-DQA1* allele was chosen for DNA typing. RERF-LC-KJ was typed as DQA1* 0103/0301 (lane 1), whereas human fetal lung implant was typed as DQA1* 0101/0401 (lane 2). Analyses of the human lung implants histologically showing RERF-LC-KJ metastasis (lanes 3 and 4) reveal that the human lung implants mainly contain RERF-LC-KJ DNA, confirming metastasis of inoculated lung cancer to human lung. Likewise, we confirmed metastasis in the case of RERF-LC-AI, although the data are not shown.

Orthotopic implantation of human tumor cells in nude mice is currently the method of choice for assessment of metastatic potential,[11-18] but only the metastasis to mouse organs can be examine in these mice. In the present study, we have established a novel experimental system to analyze metastatic potential of human cancer to human organs. Using this system, interesting metastatic features of cancer cells have been revealed. For example, RERF-LC-KJ cells, which were found to be totally non-metastatic to the lung of either SCID mice or nude mice, metastasized to the implanted human fetal lung in about 40% of the SCID mice. This result may suggest the involvement of species-specific cell interaction mechanisms in lung cancer metastasis to lung. In contrast, RERF-LC-AI seems to have lost species-specificity, because it metastasized to the lung of both human and mouse at a high frequency. Since RERF-LC-KJ cells can metastasize to the mouse lymphnode, the species-specific adhesion molecules may not be involved in the interaction with lymphnode tissue. The absence of metastasis to human adult lung implants may be due to unfavorable conditions for the adherence of cancer cells to adult lung, which retains only bronchial structural but lacks alveolar structures. Another possibility is poor angiogenesis in the implanted adult tissues.

So far we have confirmed successful implantation in SCID mice of various human epithelial tissues other than human lung tissues.[19] Thus, these SCID-human chimeric mouse systems have potential for the study of various aspects of human cancer metastases. For example, we can substantiate the "seed and soil" theory[20] by analyzing the specificity of interaction between cancer cells and various normal tissues in SCID mice. Molecular events involved in human-human cell interaction can also be examined in these *in vivo* models. Furthermore, we can use these chimeric mice to develop new modalities for prevention of human cancer metastasis, including immunological therapy, since the human immune system can also be transferred to SCID mice.[21-23]

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