Orthotopic Reconstitution of Human Small-Cell Lung Carcinoma after Intravenous Transplantation in SCID Mice

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Abstract. We have constructed an orthotopically reconstituted model of human small-cell lung carcinoma (SCLC) by intravenous transplantation in severe combined immunodeficient (SCID) mice. Two human SCLC xenografts, H-69 and Lu-130, were disaggregated and injected through the tail vein of SCID mice. Human SCLCs were orthotopically reconstituted with multi-focal lung tumor growth in all SCID mice after intravenous injection of 5 x 10^6 tumor cells per mouse. The heart and liver were also seeded with actively growing SCLC. This orthotopic reconstitution model of human SCLC in SCID mice should be useful for further studies on the biological behavior and treatment of human SCLC.

Chemotherapy is the main modality of treatment for patients with small-cell lung carcinoma (SCLC), and many combinations of chemotherapeutic regimens have been attempted (1). However, only a small minority of patients is able to achieve long-term survival because of the rapid growth and high rate of recurrence of SCLC (2). Although new regimens and agents for the treatment of SCLC are currently being tested, the lack of a suitable rodent model has impeded the investigation of new therapeutic strategies. In this study, we developed a novel model of human SCLC, which was orthotopically reconstituted in severe combined immunodeficient (SCID) mice.

Materials and Methods

Mice. Male SCID mice with a CB-17 genetic background were kindly supplied by Dr. T. Nomura, Central Institute for Experimental Animals, Kawasaki, Japan. Male nude mice with a BALB/cA genetic background were purchased from CLEA Japan Inc., Tokyo. They were maintained under specific pathogen-free conditions using an Isorack™, and fed on sterile food and water ad libitum at the experimental animal center, School of Medicine, Keio University. Six- to eight-week old mice weighing 20-22 g were used for the experiments.

Human small-cell lung carcinoma strains. H-69 was established as a cultured cell line of human SCLC at the National Cancer Institute, Bethesda, MD, and was transplanted into nude mice at the School of Medicine, Keio University. Lu-130 was established at the Pathology Division, National Cancer Center Research Institute, Tokyo, as a serially transplantable human SCLC in nude mice. Both xenografts are maintained in our Institute by serial transplantation subcutaneously in nude mice (3,4).

Orthotopic reconstitution of human SCLC in nude and SCID mice. Tumors in the exponential phase of subcutaneous growth in nude mice were resected aseptically, necrotic tissues were cut away, and remaining intact tumor tissues were scissors-minced as finely as possible in Hanks' balanced salt solution containing 100 IU penicillin and 100 mg streptomycin per ml (Hanks’ solution). After incubation for 30 min at 37°C with an enzyme cocktail containing 0.02% collagenase (Worthington Biochemical Corporation, NJ), 0.05% pronase (Boehringer Mannheim GmbH Biochemica, Germany) and 0.02% DNase (Boehringer), the homogenates were passed through a stainless steel mesh (200目), and the filtrates were washed once in RPMI-1640 (Nissui Pharmaceutical Corporation, Tokyo) medium containing 10% fetal calf serum. The filtrated homogenate was then centrifuged for 10 min at 3,000 rpm. The dissociated tumor cells were then suspended in Hanks’ solution, and the concentration of viable cells in the suspension was determined by the trypan blue dye exclusion test. After centrifugation, the tumor cells were resuspended at concentrations of 2.5 x 10^6, 10^5 and 10^4 viable cells/ml. Two hundred microliters of the tumor-cell suspension per mouse, equivalent to 5 x 10^6, 10^5 and 10^4 viable tumor cells, were injected into nude and SCID mice intravenously through the tail vein via a 27-gauge needle. The mice were sacrificed on days 7, 14 and 21 after tumor-cell injection, and the lungs, heart, liver and other main organs were removed and processed for routine histological examination after careful macroscopic observation.

Results

Table I demonstrates the percent of SCID mice in which orthotopic reconstitution of SCLC was observed as a function of time after implantation and the numbers of implanted H-69 and Lu-130 cells. No nude mice developed orthotopica-
Figure 1. Histological views of the H-69 human small-cell lung carcinoma xenograft observed after intravenous injection in SCID mice.

a: orthotopic reconstitution in lung (H&E, X 40)
b: implantation to liver (H&E, X 40)
c: implantation to heart (H&E, X 20)
ly growing SCLC. All the SCID mice developed orthotopic SCLC on day 21 after implantation with $5 \times 10^6$ H-69 cells.

Furthermore, in the case of implantation of $5 \times 10^6$ H-69 cells, orthotopically reconstituted SCLC (Figure 1a) was observed in all the SCID mice from day 14. Multi-focal tumors were found to be growing in both lungs. In addition, implantation to the liver (Figure 1b) and heart (Figure 1c) was observed in two of four mice on day 21. No SCID mice developed orthotopic reconstitution after implantation with $5 \times 10^6$ H-69 cells.

Lu-130 was orthotopically reconstituted in lungs of 2/4 mice on day 14 and 4/4 mice on day 21 when $5 \times 10^6$ cells were injected. Multi-focal tumors of Lu-130 were found to be growing in both lungs.

When the implanted SCID mice were observed for more than 21 days, tumor deaths were often encountered. All the SCID mice developed orthotopic reconstitution on day 21 after implantation of $5 \times 10^6$ cells of both strains. Thus the optimal cell number for injection was estimated to be $5 \times 10^6$ cells, and the optimal evaluation date was considered to be day 21.

**Discussion**

McLemore et al (5) reported that human lung cancer cells suspension implanted orthotopically into the bronchi of nude mice had higher take rates than those in currently-used subcutaneous implantation, suggesting that the orthotopic environment in nude mice would be an important factor for the growth of xenograft human tumors.

Recently, it has been reported that SCID mice, which congenitally lack functional T- and B- lymphocytes (6), allow the development of human leukemia, which shows a distribution similar to that in the clinical course of the disease (7). From a clinical viewpoint, SCLC has many characteristics, such as a diffuse growth pattern and rapid spread to other organs, which resemble hematogenic malignancy rather than other types of human lung carcinoma. In this study, we also used SCID mice and established a new rodent model of human SCLC, which allowed diffuse distribution of lung tumors and implantations to other organs. In the initial experiments, we used mice to implant the same SCLC, although none developed orthotopic SCLC, even when $5 \times 10^6$ cells were transferred. This result is consistent with recent work from other laboratories, indicating that SCID mice allow higher rates of growth and metastasis of xenografted human tumors in comparison with nude mice (8, 9). Our model using orthotopic reconstitution of SCLC in SCID mice seems to be the clinically relevant rodent model of SCLC. This model is complementary to another model of SCLC which we have developed utilizing the thoracotomy method to implant histologically intact lung tumor tissue into the lungs of nude and SCID mice with resulting local growth, opposite lung metastases and distant metastases (10).

Although combined chemotherapeutic regimens in current use achieve a high response rate of about 75%, the prognosis of patients with SCLC is still poor (2). The SCID mouse model with orthotopic reconstitution of human SCLC described here and the orthotopic implant model utilizing intact tissue mentioned above (10) should facilitate further studies on the treatment and biology of human SCLC.

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**References**


**Table 1. Orthotopic reconstitution of H-69 and Lu-130 in nude and SCID mice.**

<table>
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<td></td>
<td>Day 7</td>
</tr>
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<td>H-69</td>
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<td>0/4$^*$</td>
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<tr>
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<tr>
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$^*$Data are shown as number of mice with lung reconstitution / number of mice evaluated.

$^b$Implantations of SCLCs to liver and heart were observed on Day 21 in two of four mice implanted with $5 \times 10^6$ tumor cells.


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