Site-Specific Chemosensitivity of Human Small-Cell Lung Cancer Growing Orthotopically Compared to Subcutaneously in SCID Mice: The Importance of Orthotopic Models to Obtain Relevant Drug Evaluation Data

TSONG-HONG KUO1, TETSURO KUBOTA1, MASAHIKO WATANABE1, TOSHIHARU FURUKAWA1, SUGURU KASE1, HIROKAZU TANINO1, YOSHIRO SAIKAWA1, KYUYA ISHIBIKI1, MASAKI KITAJIMA1 and ROBERT M. HOFFMAN2,3

1Department of Surgery, School of Medicine, Keio University, 35 Shinanomachi, Shinjuku-ku, Tokyo 160 Japan;
2AntiCancer Inc., 5325 Metro Street, San Diego, CA 92110;
3Laboratory of Cancer Biology, University of California, San Diego, La Jolla, CA 92037-0609, U.S.A.

Abstract. We have developed a novel in vivo model of human small-cell lung carcinoma (SCLC) using orthotopic reconstitution by injecting human SCLC in the tail vein of severe combined immunodeficient (SCID) mice whereby the SCLC grows in the lung and other organs. Cisplatin (DDP) had significant antitumor effects on the SCLC growing orthotopically in the lung whereas mitomycin C (MMC) did not, thereby reflecting the clinical situation. However, the opposite effects were found when the SCLC was growing subcutaneously, where the tumors responded to MMC and not to DDP. This suggests that the tumors growing orthotopically reflect the clinical effects of drugs on human SCLC more closely than the tumors growing subcutaneously. Therefore, this orthotopic reconstitution model of human SCLC in SCID mice is thought to be useful for studies on the treatment of human SCLC and emphasizes the need for orthotopic models for relevant cancer drug evaluation.

Chemotherapy is the main modality of treatment for small-cell lung carcinoma (SCLC), and many combinations of chemotherapeutic regimens have been attempted (1). However, the prognosis is still poor because of the rapid growth and high rate of recurrence of SCLC (2). Furthermore, the lack of a suitable rodent model for chemosensitivity assays has impeded the investigation of new therapeutic strategies. We have developed a novel model of human SCLC, which was orthotopically reconstituted in the lung after intravenous injection of human small-cell lung carcinoma into severe combined immunodeficient (SCID) mice (3). The present paper describes the results of cancer chemotherapy for human SCLC reconstituted orthotopically in the lungs and other organs of SCID mice compared to subcutaneously growing SCLC.

Materials and Methods

Mice. Male SCID mice with a CD-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. The animals were maintained under specific pathogen-free conditions using an Isorack™, and fed on sterile food and water ad libitum in the experimental animal center of Keio University, Sist - to eight-week-old mice weighing 20–22 g were used for the experiments.

Human small-cell lung carcinoma cell line. Lu-130 was established at the Pathology Division, National Cancer Center Research Institute, Tokyo, as a serially transplantable human SCLC in nude mice. The xenografts are maintained in Keio University by serial subcutaneous transplantation in nude mice (4, 5).

Anticancer drugs. Mitomycin C (MMC) was purchased from Kyowa Hakko Kogyo Co., Ltd., Tokyo and cisplatin (DDP) was purchased from Bristol - Myers Squibb K.K., Tokyo.

Orthotopic reconstitution of human SCLC in SCID mice. Exponentially growing tumors in the subcutaneous tissue of nude mice were resected aseptically, necrotic tissues were cut away, and the remaining intact tumor tissues were scissor-mixed as finely as possible in Hanks’ balanced salt solution containing 100 IU penicillin and 100 μg 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 μm), and the filtrates were washed once in RPMI-1640 (Nissui Pharmaceutical Corporation, Tokyo) medium containing 10% fetal calf serum. The filtered homogenate was then centrifuged for 10 min at 3,000 rpm 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 dissociated tumor cells were resuspended at a concentration of 2.5 x 10⁷ viable cells/ml. Two hundred microliters of the tumor-cell suspension per mouse, equivalent to 5 x 10⁷ viable tumor cells, were injected into SCID

Correspondence to: Tetsuro Kubota, M.D., Dept. of Surgery, School of Medicine, Keio University, 35 Shinanomachi, Shinjuku-ku, Tokyo 160 Japan.

Key Words: Human small-cell lung carcinoma, orthotopic transplant, site-specific cancer chemotherapy, SCID mouse.

0250-7005/93 $2.00 + .40
mice intravenously through the tail vein via a 27-gauge needle. The mice were sacrificed on day 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.

**Chemosensitivity testing of orthotopically growing SCLC cells in SCID mice.** Seven days after intravenous injection of tumor cells, the mice were randomized into control and treatment groups, and the treatment was initiated. Six milligrams of MMC per kg were dissolved in 0.2 ml of 0.9% NaCl per 20 g body weight and administered intraperitoneally. DDP was also administered intraperitoneally at a dose of 9 mg/kg, which was equivalent to 0.35 ml solution per 20 g body weight. These administered doses had been determined to be the maximum tolerated doses (MTDs) in our previous studies (5). The mice were observed three times per week for 3 weeks after tumor-cell injection, then sacrificed for macroscopic and histological observation on day 21. Statistical analysis of the data was performed according to the chi-squared test.

**Chemosensitivity testing of subcutaneously growing SCLC tumors in nude and SCID mice.** Tumors resected from nude mice were scissor-cut into 3 x 3 x 3 mm pieces and inoculated into the subcutaneous tissue on either side of the backs of nude and SCID mice. The length and width of the tumors were measured with sliding calipers three times by the same person. The tumor weight was estimated according to the formula: tumor weight (mg) = length (mm) x width (mm)^2. When the tumors reached 100-300 mg, usually 2-3 weeks after tumor inoculation, tumor-bearing mice were randomized into control and treatment groups, and the treatment was initiated. Drugs were administered using the same schedule as that for the chemosensitivity assay of orthotopically growing tumors. The mice and tumors were observed three times a week for 3 weeks after the initial treatment. On day 21 after treatment, the mice were sacrificed and the tumors that had grown subcutaneously in each mouse were removed and weighed. The antitumor effect of the drugs was evaluated by the T/C ratio (%), where T was the actual tumor weight of the treated group and C the actual tumor weight of the control group on day 21 after the treatment. Statistical analysis of the data was performed according to Student's t test.

**Results**

**Chemosensitivity of orthotopically growing SCLC in SCID mice.** Table I shows the antitumor activity of MMC and DDP against Lu-130 orthotopically reconstituted in SCID mice. Since it was difficult to count the exact number of implanted nodules of SCLC in the lungs because of fusion of the nodules, we used the number of mice with lung implantation of SCLC as a quantitative indicator. DDP reduced the incidence of orthotopic reconstitution of Lu-130 tumor cells from 10/10 to 3/10 mice (p < 0.01), whereas all SCID mice treated with MMC had growing human SCLC tumors in the lungs. Upon pathological examination, no significant difference was observed in the histological appearance of the lungs in the mice treated with DDP in comparison to the control (Figure 1).

**Table I. Chemosensitivity testing of Lu-130 human small-cell lung carcinoma orthotopically growing in SCID mice.**

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Treatment</th>
<th>Reconstitution in lung</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lu-130</td>
<td>Control</td>
<td>10/10</td>
</tr>
<tr>
<td></td>
<td>DDP</td>
<td>3/10</td>
</tr>
<tr>
<td></td>
<td>MMC</td>
<td>10/10</td>
</tr>
</tbody>
</table>

Five million tumor cells were injected intravenously on Day 0, and mice were sacrificed on Day 21.

*Data are shown as number of mice with lung reconstitution or implantation to the other organs / number of mice evaluated.

*Cisplatin was administered ip at a dose of 9 mg/kg on Day 7.

*Mitomycin C was administered ip at a dose of 6 mg/kg on Day 7.

*Statistically significant relative to control by chi-squared test: p<0.01.
MMC and DDP against Lu-130 implanted subcutaneously into nude and SCID mice. The subcutaneous growth of 
Lu-130 was significantly inhibited in the mice treated with MMC (p < 0.01), whereas no significant reduction was 
observed in the nude and SCID mice treated with DDP (Table II). The chemosensitivity of the subcutaneously 
growing SCLC is in direct contrast to the tumors growing orthotopically in the lung, which in the case of Lu 130 respond to 
DDP but not MMC as described above. No significant difference was observed between nude and SCID mice when 
the subcutaneously implanted SCLC was used for evaluating the effect of DDP and MMC on Lu-130.

Discussion

It has been reported that SCID mice, which congenitally lack functional T and B lymphocytes (9), allow higher rates of 
growth and metastasis of xenografted human tumors in comparison with nude mice (10, 11). We have previously 
developed a new rodent model of human SCLC using SCID mice, which allows diffuse growth of SCLC in the mouse 
lungs and implantation to other organs, whereas implantation of SCLC was not observed in nude mice (3). In this study, we 
utilized this model to develop a new in vivo chemosensitivity assay of SCLC.

Human tumor xenografts transplanted subcutaneously into nude mice are reported to be somewhat more sensitive to 
chemotherapeutic drugs than the corresponding patients, in particular to MMC (12, 13). In this study, MMC also 
demonstrated marked antitumor effects on Lu-130 implanted into the subcutaneous tissue of nude and SCID mice, 
although MMC is not regarded as a standard treatment for human SCLC in the clinical setting (14). On the other hand, 
the efficacy of DDP against Lu 130 was limited against subcutaneously growing SCLC in nude mice and SCID mice, 
although DDP is one of the key agents for treatment of SCLC (14). However, when we used the SCID - mouse model of 
orthotopic reconstitution for the chemosensitivity assay, DDP reduced the incidence of orthotopic reconstitution of 

Lu-130, whereas MMC had no apparent effect on the orthotopically - growing tumors. These data suggest that the 
orthotopically reconstituted model of SCLC in SCID mice is potentially a clinically relevant model of human SCLC.

Iigo et al (15) have reported that some drugs showed marked differences in antitumor activity against subcutaneous tumors on the one hand and liver metastases on the other in a nude mouse model, suggesting that the chemosensitivity of tumors to drugs may depend on the tumor growth site. In the present study, DDP and MMC demonstrated differences in antitumor activity against SCLC growing at different sites. In our previous report (16), we observed that 5-fluorouracil showed significant antitumor activity in preventing a liver metastasis in a liver metastatic model of human colon cancer transplanted intrasplenically into nude mice, whereas 5-fluorouracil did not show apparent effects against subcutaneously growing tumors of the same colon tumor cell line. These results could be due to the different pharmacokinetics of the drugs in different organs, 
including absorption, distribution, metabolism and excretion, as well as different micro-environments (26, 27).

Although new regimens and agents for the treatment of SCLC are currently being tested, the prognosis of SCLC is 
still poor and the lack of a suitable rodent model for chemosensitivity testing has impeded the investigation of new 
therapeutic stategies. Since no orthotopic reconstitution was observed in nude mice (3), SCID mice were thought to be 
useful for evaluation of promising agents for treatment of human SCLC. This new SCID mouse model using orthotopic 
reconstitution of human SCLC should facilitate further studies on the treatment of human SCLC, in addition to the 
other orthotopic transplant models developed in our laboratories (17 - 25).

Acknowledgements

The authors are grateful to Dr. T. Nomura, Central Institute for Experimental Animals, for kindly supplying SCID mice. The authors are also grateful to Ms. M. Nagata and Ms. K. Tokuno for their excellent technical assistance.
References


Received December 30, 1992
Accepted March 8, 1993