Response of Small-Cell Lung Cancer Xenografts to Chemotherapy: Multidrug Resistance and Direct Correlates

M. F. Poupon, F. Arvelo, A. F. Goguel, Y. Bourgeois, M. Jacrot, N. Hanania, R. Arriagada, T. Le Chevalier*

Background: Patients with small-cell lung carcinomas (SCLCs) initially respond to combination chemotherapy. Only a few benefit in terms of long-term survival because most relapse. Such outcome may be attributable to development of multidrug resistance. Purpose: The response of SCLC to chemotherapy was examined in terms of (a) patient survival, (b) drug sensitivity of tumors in patients and of tumor xenografts in nude mice, and (c) expression of multidrug resistance gene MDR1 and GST-π gene. Methods: Tumor samples obtained from seven untreated patients and from one patient both before and after chemotherapy were transplanted into nude mice. The patients were treated with a combination of cyclophosphamide (C'), cisplatin (C), doxorubicin (A), and etoposide (V) (C'CAV) or C'AV and radiotherapy. Drug sensitivity of SCLCs was tested in nude mice that had received tumor xenografts from these seven patients. The expression of MDR1 and GST-π genes was assessed in the mRNA extracted from xenografts by Northern blot analysis. P-glycoprotein was quantified by enzyme immunoassay. Results: The patients' responses to C'CAV closely correlated with those of the corresponding xenografts. The tumors of the two patients who showed long-term survival after C'CAV completely regressed when they were transplanted into nude mice and subsequently treated with C'CAV. Despite initial complete response, the remaining five patients died during year 1. A high percentage of mice receiving the tumor grafts from these five patients showed only partial tumor regression after C'CAV treatment. The MDR1 transcript was detected in all five of these xenografts. Four of five xenografts were from untreated patients, and the fifth was from a treated patient. MDR1 mRNA expression was absent in the tumor of this fifth patient before chemotherapy, but both the mice receiving the corresponding xenograft and the patient showed expression of MDR1 after C'CAV treatment. MDR1 mRNA expression was absent in the tumor xenografts obtained from two patients with long-term survival. Expression of P-glycoprotein correlated with MDR1 mRNA expression. All xenografts except one expressed the GST-π gene. Conclusions: The absence of MDR1 gene expression during chemotherapy for SCLC indicates a favorable prognosis, gene expression is often coincident with ineffective chemotherapy, and tumor xenografts can be appropriately used to predict response to chemotherapy. Implications: Failure of chemotherapy to control SCLC seems to be related to an acquired multidrug resistance involving the MDR1-mediated mechanism. Therapeutic benefit could therefore be expected from chemotherapy combined with inhibitors of MDR1. [J Natl Cancer Inst 85:2023–2029, 1993]

Notes

Affiliations of authors: T. Mitsudomi, T. Oyama, T. Osaki, R. Nakanishi, T. Shirakusa, Department of Surgery II, University of Occupational and Environmental Health, Kitakyushu, Japan. T. Kusano, Department of Surgery II, Fukuoka University, School of Medicine, Japan. Correspondence to: Tetsuya Mitsudomi, M.D., Department of Surgery II, University of Occupational and Environmental Health, Isieigaoka 1-1, Yahatanishi-ku, Kitakyushu 807, Japan. Supported by Grant-in-Aid (04543351) from the Ministry of Education, Science, and Culture of Japan and by grants from the Fukuoka Cancer Society and the Kaibara Morikazu Medical Science Promotion Foundation. We thank Dr. Adi F. Gazdar and Dr. David P. Carbone for pertinent comments. Manuscript received June 7, 1993; revised August 26, 1993; accepted September 13, 1993.

Small-cell lung carcinomas (SCLCs) are highly responsive to various chemotherapeutic treatments. Long-term survival, however, is achieved in only a small percentage of patients with SCLC. What is it that distinguishes a curable SCLC from an incurable one? If we assume that individual SCLCs respond differently to chemotherapy, can this difference be based on a difference in the level of the expression of genes known to be responsible for a
multidrug-resistant phenotype? In other words, is the chemosensitivity of the tumor dependent on the expression of such genes? These questions can be broached in studying human tumors. The chemosensitivity of such tumors can be accurately evaluated in vivo after tumor tissue samples obtained from patients are transplanted into animal hosts. Xenografting techniques have enabled researchers to grow human tumors in immunosuppressed mice. Such techniques have made it possible to study in vivo the antitumor effects of agents used in cancer therapy and to conduct cytogenetic, histologic, or genetic studies on the tumors (1,2).

The results obtained from such studies can be considered valid when particular tumors treated with a given chemotherapy protocol respond in a similar manner in humans and in nude mice. As Bellet et al. (3) postulated, it is possible to assess the response to a given anticancer agent of a panel of human tumors of the same histologic type that have been transplanted into nude mice. By a comparison of the results obtained with the same tumor in the two hosts, it may be possible to assess the similarity of results and to determine to what extent human results can be predicted from results obtained in mice.

This report describes our results with such an approach in eight SCLCs obtained from seven untreated patients. These patients were subsequently treated according to previously reported protocols (4-7), and their response to therapy was already known. By xenografting samples of these tumors in nude mice, we could study the chemosensitivity of these tumors as well as their drug resistance phenotype as a function of the expression of the MDR1 gene (also known as P7Y1) and the GST-π gene (also known as GSTP1). Overexpression of the MDR1 gene encoding for P-glycoprotein, a transmembrane protein responsible for accelerated efflux of a large panel of cationic drugs, causes multidrug resistance in very diverse types of cancers and in some normal tissues, including the adrenal cortex, medulla kidney, liver, brain capillary endothelial cells, and lung (8,9). MDR1 gene overexpression in SCLC remains a controversial subject (10-14). Glutathione S-transferases (GSTs) are also implicated in the protection of cells against cytotoxic and carcinogenic agents (15), and the acidic class π protein was more than not the most abundant form of GST found in all the human tumors studied, including lung, colon, bladder, and breast carcinomas. Among the multidrug resistance mechanisms established to date, namely those related to topoisoerase function (16), and among the new molecular determinants, such as MRP (multidrug-related proteins) which were initially discovered in multidrug-resistant SCLC cell lines (17), we have focused our study on the MDR1-dependent mechanism of resistance because of the clinical and therapeutic possibilities of reversing this resistance in the not too distant future.

Materials and Methods

Tumors

The tumors were obtained by biopsy from patients with SCLCs. The tumor tissue from the patient was immediately transplanted into the nude mice (2). Cytogenetic studies of xenografts have revealed a human karyotype. Table 1 summarizes the main characteristics of each tumor. Sufficient tumor material was available from two patients. This was kept frozen so that it was possible to compare gene expression in patient tumor samples with that in the xenografts derived from these patients. A kidney carcinoma, transplanted into nude mice, was used as a control reference for MDR1 overexpression. All tumor materials were stored in liquid nitrogen.

Patients

Written, informed consent was obtained from each subject. These investigations were performed after approval by a local institutional review board.

Animals

Swiss (nu/nu) female mice, 6-8 weeks old, were purchased from IFFA-Credo (Lyon, France). The animals were maintained under specific pathogen-free conditions. Their care and housing were in accord with institutional guidelines as put forth by the Ministère de l’Agriculture et de la Fôret, Direction de la Santé de la Protection Animale, Paris, France.

Experimental Procedures

Xenografts were established by the subcutaneous implantation of the human tumor fragments into the scapular area of nude mice under ether anesthesia for a short period of time. Tumors appeared after 4-24 weeks (first passage). After reaching a volume of 500 mm³, they were serially transplanted from mouse to mouse (from three to 10 times) as previously described (2). When their local tumors reached a 1.6 cm³ volume, the mice were killed by prolonged exposure to ether anesthesia.

For chemotherapy assays, the mice were randomly divided into groups of 10-20 animals and tumors were transplanted in a similar manner. The tumor-bearing mice were treated with chemotherapeutic agents as soon as the mean diameter of their tumors reached 5-8 mm (or tumor volume of 60-250 mm³). Mice received the C’AV regimen, which consisted of cyclophosphamide (C) (Endoxan-Asta; Laboratoires Sarget, Mérignac, France), cisplatin (C) (Cisplatyl; Laboratoires Roger Bellon, Neuilly-sur-Marne, France), doxorubicin (A) (Adriablastine; Laboratoires Roger Bellon), and etoposide (V) (Vépéside; Sandoz, Rouen, France). C’AV was used for treating the tumor donors of SCLC-10 and SCLC-74A only, not the mice. All agents were injected by the intraperitoneal route. On day 1, the mice received 6 mg/kg doxorubicin and 8 mg/kg etoposide; on day 2, they received 8 mg/kg etoposide and 3 mg/kg cisplatin; on day 3, they were given 8 mg/kg etoposide and 50 mg/kg cyclophosphamide; on day 4, they were given 50

Table 1. Characteristics of the human tumors and duration of patient survival

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Sex/age, y. of patients</th>
<th>Location of tumor</th>
<th>Histologic type</th>
<th>Previous treatment</th>
<th>Survival duration, mo*</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCLC-10</td>
<td>Male/47</td>
<td>Primary site</td>
<td>Oat-cell</td>
<td>None</td>
<td>&gt;142</td>
</tr>
<tr>
<td>SCLC-61</td>
<td>Male/55</td>
<td>Metastasis</td>
<td>Oat-cell</td>
<td>None</td>
<td>30</td>
</tr>
<tr>
<td>SCLC-41</td>
<td>Male/55</td>
<td>Metastasis</td>
<td>Oat-cell</td>
<td>None</td>
<td>4</td>
</tr>
<tr>
<td>SCLC-74A</td>
<td>Male/55</td>
<td>Metastasis</td>
<td>Oat-cell</td>
<td>None</td>
<td>8 (total)</td>
</tr>
<tr>
<td>SCLC-74B</td>
<td>Male/55</td>
<td>Metastasis</td>
<td>Intermediate</td>
<td>C’AV†</td>
<td>4</td>
</tr>
<tr>
<td>SCLC-30</td>
<td>Male/52</td>
<td>Metastasis</td>
<td>Intermediate</td>
<td>None</td>
<td>4</td>
</tr>
<tr>
<td>SCLC-6</td>
<td>Male/48</td>
<td>Metastasis</td>
<td>Intermediate</td>
<td>None</td>
<td>8</td>
</tr>
<tr>
<td>SCLC-75</td>
<td>Male/47</td>
<td>Primary site</td>
<td>Intermediate</td>
<td>None</td>
<td>10</td>
</tr>
</tbody>
</table>

*Duration of patient survival.
†Death due to the treatment.
‡Tumor samples obtained from the same patient before (A) and after (B) treatment.
§Cyclophosphamide + doxorubicin + etoposide.
mg/kg cyclophosphamide; and on day 5, they were again given 50 mg/kg cyclophosphamide. The mice in the control group received injections of isotonic saline solutions.

We monitored tumor growth by measuring the tumor diameter with a caliper. We calculated the tumor volume (V) by the following equation:

\[ V = \frac{a^2 \times b}{2}, \]

where \( a \) is the width of the tumor in millimeters (large diameter) and \( b \) is the length of the tumor in millimeters (small diameter) (3).

The relative tumor volume (RTV) was calculated by the following equation:

\[ RTV = \frac{V_t}{V_0}, \]

where \( V_t \) is the mean tumor volume in cubic millimeters at any time given and \( V_0 \) is the mean initial tumor volume in cubic millimeters at the start of treatment; computation of the RTV allowed us to compare the tumor growth despite initial differences in volume.

The drug effect was expressed as the tumor growth inhibition, evaluated between day 10 and day 20. Tumor growth inhibition was calculated by the following equation:

\[ \left[ 100 - \frac{(V_t/C)}{V_0} \right] \times 100, \]

where \( T \) is the mean RTV of the treated tumors and \( C \) is the mean RTV of the tumors in the control group.

### RNA Extraction and Northern Blot Analysis

Tissue specimens were immediately cut into pieces, immersed in liquid nitrogen, and then kept at -70°C until they were processed. Solid tumors were pulverized prior to RNA extraction. Total RNA was extracted with guanidium isothiocyanate and layered over cesium chloride (5.7 M) in a solution of sodium acetate (25 mM [pH 5.0]) (18). Gradients were centrifuged overnight in a Beckman SW41 rotor (Beckman Instruments, Inc., Fullerton, Calif.) at 33,000 rpm. Total RNA (20 µg) was electrophoresed in formaldehyde gels (1%) and transferred to nylon Hybond-N filters (Amersham Corp., Arlington Heights, Ill.). The transfer was performed essentially according to the procedure of Maniatis et al. (19). Baked filters were prehybridized for 18-24 hours at 42°C. The hybridization buffer contained 50% formamide, 5X SSC (1X 0.15 M sodium chloride, 0.015 M sodium citrate, and 50 mM Na2HPO4-NaH2PO4 [pH 6.5]), Denhardt’s solution (0.02% bovine serum albumin, 0.02% polyvinylpyrrolidone, and 0.02% Ficoll), and 100 mg/mL sonicated salmon sperm DNA. The probes were labeled with [32P]deoxyxycytidine triphosphate by a random priming labeling system according to the supplier’s recommendations (Boehringer-Mannheim Biochemicals, Indianapolis, Ind.) (specific activity, 10⁸ cpm/mg DNA). The following probes were utilized: MDR1 pUC9 (20), GST-κ PGEM4 (21), and GAPDH (i.e., glyceraldehyde-3-phosphate-dehydrogenase) pBR322 (22). The final wash of filters was carried out three times at room temperature in 2X SSC and 0.1% sodium dodecyl sulfate at 55°C for 1 hour. Autoradiography was performed at -70°C on Agfa-Gevaert-Curix P2 films (Agfa, Leverkusen, Fed. Republic of Germany).

### Evaluation of P-glycoprotein Expression by Enzyme Immunoassay

SCLC cell lines were established in short-term cultures from xenografts. Cells were cultivated in RPMI-1640 medium supplemented with 10% fetal calf serum. Tests were performed in enzyme immunoassay microtiter plates. Briefly, 2.5 X 10⁵ cells were seeded in duplicate wells and diluted twofold serially three times. After centrifugation at 600g, the cells were fixed in a 3% paraformaldehyde-phosphate-buffered saline solution. The cells were incubated with JSB1 antibody (Sanbio, Uden, The Netherlands) at a 1:25 dilution in phosphate-buffered saline. The antibody binding was measured using an avidin-biotin peroxidase complex test (Vectastain ABC kit; Vector Laboratories, Inc., Burlingame, Calif.). Cells were dissolved in 50 µL dimethyl sulfoxide, and the optical density (OD) of dimethyl sulfoxide-treated cell extracts was read on a Titertek Multiskan Photometer (J.BioSA., Courtaboent, France) at a wavelength of 492 nm. We calculated the differences between the OD in the wells containing the cells incubated with the JSB1 antibody and the OD in the wells containing the cells without antibody (ΔOD). These ΔOD values were then transformed to arbitrary units: One unit represents the ΔODu of the cell extract with the lowest expression of P-glycoprotein; ΔOD of the other samples are reported as ΔODu and are presented as units of P-glycoprotein expression.

### Results

**Response of Xenografts to Chemotherapy**

Table 2 summarizes the effects of the combination chemotherapy C'CAV on the growth of xenografted SCLCs. Two parameters were taken into account: complete regressions and partial regressions. Complete regressions were usually achieved by days 9-13, and...

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Treatment*</th>
<th>Tumor-doubling time, d</th>
<th>No. of surviving mice†</th>
<th>No. of responses§</th>
<th>% tumor growth inhibition$ (cures†)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCLC-10</td>
<td>Control</td>
<td>11</td>
<td>19/20</td>
<td>93</td>
<td>17/20</td>
</tr>
<tr>
<td>SCLC-61</td>
<td>Combination chemotherapy</td>
<td>-</td>
<td>9/10</td>
<td>100</td>
<td>9/10 (60%)</td>
</tr>
<tr>
<td>SCLC-41</td>
<td>Control</td>
<td>14</td>
<td>8/10</td>
<td>58</td>
<td>0/10 (60%)</td>
</tr>
<tr>
<td>SCLC-74A¶</td>
<td>Control</td>
<td>15</td>
<td>10/12</td>
<td>60</td>
<td>0/10 (60%)</td>
</tr>
<tr>
<td>SCLC-74B¶</td>
<td>Combination chemotherapy</td>
<td>11</td>
<td>13</td>
<td>83</td>
<td>0/10 (60%)</td>
</tr>
<tr>
<td>SCLC-61</td>
<td>Control</td>
<td>7</td>
<td>15/15</td>
<td>83</td>
<td>0/10 (60%)</td>
</tr>
<tr>
<td>SCLC-75</td>
<td>Combination chemotherapy</td>
<td>12</td>
<td>13/17</td>
<td>65</td>
<td>0/10 (60%)</td>
</tr>
</tbody>
</table>

*See "Experimental Procedures" in the "Materials and Methods" section for explanation of treatment.
†Number of mice surviving/total number of mice treated after 60 days. Cures correspond to the number of mice that did not have any tumor burden after 90 days/total number of mice treated.
§Number of mice that showed a significant tumor growth inhibition/total number of mice per group (up to 50% of growth inhibition between day 10 and day 20).
$Calculated as \[100 - \left(\frac{T}{C}\right)\] (the mean size of the treated tumors divided by the mean size of tumors of the control group) \times 100.
¶Tumors had completely regressed.
¶Tumor samples obtained from the same patient before (A) and after (B) treatment.
partial regressions (up to 50% of tumor growth inhibition) were assessable between day 10 and day 20. Table 2 shows the mean percentages of tumor growth inhibitions. As indicated, the SCLC-10 and SCLC-61 xenografts exhibited complete regression, recorded as early as day 5 (17 of 19 treated mice and nine of 10 treated mice, respectively). No recurrence was observed up to 3 months later. Complete regressions in four of 13 mice and partial regressions in nine of 13 mice were obtained with the SCLC-75 xenografts after C’CAV chemotherapy. Strong inhibitions of growth, yet no complete regressions, were observed in 13 of the 15 SCLC-6 xenograft-bearing mice. However, recurrences of the SCLC-75 and SCLC-6 tumors were constant; the tumors regrew as early as 20 days after treatment. All eight SCLC-41 xenografts showed a weak therapeutic response, near 50% of growth inhibition. The SCLC-74 xenografts obtained from the same patient before (SCLC-74A) and after (SCLC-74B) therapy responded differently to C’CAV. Up to 50% of tumor growth inhibition was observed in 10 of 10 mice with the SCLC-74A xenograft and in six of 10 mice with the SCLC-74B xenograft.

Responses and Survival of Corresponding Patient Donors

The responses of the xenografts treated with chemotherapy were compared with the responses of the corresponding patient donors. Six patients presented with limited disease at the time of diagnosis and biopsy. The patient donors of tumors SCLC-6, SCLC-61, and SCLC-75 subsequently received C’CAV, and a complete regression of tumor foci was observed. A complete and lasting cure was achieved in the patient donor of SCLC-10; this patient was treated by surgical excision of the primary tumor followed by combination chemotherapy (C’AV) and radiotherapy. The same treatment (chemotherapy and radiotherapy) was given to the patient donor of the SCLC-74A tumor, but no cure was achieved. Mediastinal lymph node metastases occurred 4 months later. The mediastinal metastases were surgically excised, and a sample of tumor tissue was transplanted (SCLC-74B). This patient received C’AV, but this treatment failed to prevent tumor progression. The tumor response of the patient donor of SCLC-41 was not assessable because the patient died. The tumor of the last patient, the donor of SCLC-30, was resistant to C’CAV chemotherapy.

In terms of the duration of survival, two of seven patients benefited from C’CAV or C’AV therapy with long-term survival: One (the donor of SCLC-41) treated by surgery followed by chemotherapy is still alive after more than 12 years; the other (the donor of SCLC-61) treated with C’CAV and radiotherapy survived 30 months. Among the other five patients, the donors of SCLC-75 and SCLC-6, who received six cycles of C’CAV, had a relapse and died after 10 and 8 months, respectively. The patient donor of the SCLC-41 tumor xenograft died early after the first cycle of treatment. The patient donor of the SCLC-30 tumor survived only 4 months. The last patient (the donor of the SCLC-74B tumor) died 4 months after relapse.

Analysis of MDR1 and GST-π Gene Transcripts

Preparations of messenger RNA (mRNA) were analyzed for MDR1 and GST-π transcripts by Northern blot hybridization. First, we compared the MDR1 gene expression in RNA extracted from tumor samples that were obtained from the patient and frozen with that from the same tumors after growth in the nude mice (Fig. 1). This experiment was done using SCLC-30 xenografts after three passages and SCLC-41 xenografts after four passages; both were obtained from untreated patients. Signals obtained from tumor samples taken from patients appeared stronger than those obtained from tumor samples taken from mice. MDR1 mRNA overexpression was clearly observed in all cases (Fig. 1, A). Because changes could occur according to the passage number, we studied the mRNA transcripts after four different passages using the SCLC-6 xenograft. Despite some variations in signal intensity, we concluded that MDR1 was expressed in a stable manner at an intermediate level, regardless of the passage number (4, 14, 20, and 27). Fig. 1, B, permits a comparison of this level of expression to that of SCLC-75 and the kidney tumor as positive controls. Six other RNA preparations from SCLC xenografts were analyzed for MDR1 and GST-π transcripts. The MDR1 transcript (in total RNA) was undetectable in three of the eight small-cell lung tumors of human origin that were grafted into nude mice (SCLC-10, SCLC-61 [Fig. 1, C]; SCLC-74A [data not shown]). MDR1 expression became apparent in xenograft SCLC-41 (Fig. 1, A) and in xenograft SCLC-74A (Fig. 1, C) after one cycle of treatment with C’CAV given to nude mice. The MDR1 expression in SCLC-74B treated with C’CAV and SCLC-74B appear very similar. MDR1 expression was detectable in the SCLC-74B xenograft that had originated from the treated patient, low in the SCLC-41 xenograft, and moderate in the SCLC-6 and SCLC-30 xenografts; MDR1 was highly overexpressed in the SCLC-75 xenograft. These four tumors (i.e., SCLC-41, SCLC-6, SCLC-61, and SCLC-75) originated from untreated patients. This high level of expression was comparable to that of the kidney carcinoma obtained from a treated patient. All xenografts, except SCLC-41, expressed a detectable, albeit moderate, level of the GST-π gene (Fig. 1, C; Table 3).

Expression of P-glycoprotein

Using an enzyme immunoassay, we evaluated the expression of P-glycoprotein in the different SCLC cell lines derived from the corresponding tumors derived from the mice. In Table 3, the values reported correspond to arbitrary P-glycoprotein units expressed as $6.25 \times 10^4$ cells per well. SCLC-10 and SCLC-61 cells expressed the lowest levels of P-glycoprotein, while SCLC-6 and SCLC-75 cells expressed the highest levels. These four SCLC cell lines came from untreated patients. SCLC-74A cells did not significantly bind the JSB1 antibody, while SCLC-74B cells showed a high level of antibody binding.
**Table 3. Expression of MDR1 and GST-\(\pi\) genes and of P-glycoprotein in small-cell lung cancer xenografts**

<table>
<thead>
<tr>
<th>Tumor</th>
<th>MDR1 mRNA expression*</th>
<th>P-glycoprotein expression†</th>
<th>GST-(\pi) mRNA expression*</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCLC-10</td>
<td>0 (0)</td>
<td>1</td>
<td>+</td>
</tr>
<tr>
<td>SCLC-61</td>
<td>0</td>
<td>1</td>
<td>+</td>
</tr>
<tr>
<td>SCLC-41</td>
<td>+ (+)</td>
<td>ND</td>
<td>0</td>
</tr>
<tr>
<td>SCLC-74A†</td>
<td>0 (++)</td>
<td>1.5</td>
<td>+</td>
</tr>
<tr>
<td>SCLC-74B‡</td>
<td>++</td>
<td>20</td>
<td>+</td>
</tr>
<tr>
<td>SCLC-30</td>
<td>+</td>
<td>ND</td>
<td>+</td>
</tr>
<tr>
<td>SCLC-6</td>
<td>+ (+)</td>
<td>13</td>
<td>+</td>
</tr>
<tr>
<td>SCLC-75</td>
<td>++ (+)</td>
<td>14</td>
<td>+</td>
</tr>
</tbody>
</table>

*MDR1 and GST-\(\pi\) mRNA expressions in xenografts were rated visually. 0 = no visible transcript; + = trace level; and + to +++ = increasing levels. Symbols in parentheses indicate MDR1 mRNA expression after one cycle of C’CAV given to tumor-bearing nude mice.

†Expression of P-glycoprotein was evaluated by enzyme immunoassay using the JSB1 monoclonal antibody in the different SCLC cell lines derived from the corresponding xenografts. Arbitrary units are reported. One unit corresponds to the differential optical density (\(\Delta OD\)) of the cell samples with the lowest P-glycoprotein expression (\(\Delta OD_u\)). \(\Delta OD\) values of the other samples were reported as \(\Delta OD_u\). All these measurements were done on \(6 \times 10^6\) cells per well. ND = not determined.

‡Tumor samples obtained from the same patient before (A) and after (B) treatment.

**Discussion**

The group of seven patients with SCLC which we studied had a typical response to therapy. Indeed, complete tumor regressions were obtained in four patients (donors of the SCLC-10, SCLC-61, SCLC-6, and SCLC-75 xenografts). Among these patients, one (the donor of the SCLC-10 xenograft) is still alive after more than 12 years, and another (the donor of the SCLC-61 xenograft) benefited from a long-term survival. The other patients (donors of the SCLC-6 and SCLC-75 xenografts) had relapses in less than 1 year and died. The patient donors of the SCLC-30 and SCLC-74 xenografts achieved a partial response with C’CAV or C’AV chemotherapy but died 4 months later. Altogether, in this very small series, 57% of the patients (four of seven patients) had complete responses, 28% (two of seven patients) had long-term survival, and 14% (one of seven patients) had partial responses. The death of the patient, donor of the SCLC-41 xenograft, was due to treatment toxicity.

The responses of the SCLC xenografts to chemotherapy correlated with those of the respective patient donors. All the tumor xenografts exhibited substantial growth inhibition after treatment, with the exception of the SCLC-41 and SCLC-74B xenografts.
which were more resistant to treatment. Local regrowth of SCLC-74A, SCLC-74B, SCLC-75, SCLC-6, and SCLC-41 tumors was observed 2 weeks after the treatment was discontinued, while a majority of the mice with the SCLC-10 and SCLC-61 tumors were cured.

In terms of the validity of the model system, the crucial question is whether xenografts in any way retain the characteristics of the human tumor counterparts. Giovannella et al. (23) made a claim to that effect while testing the antitumor efficacy of agents generally effective in the clinic against human colorectal carcinoma, breast carcinoma, and melanoma xenografts, which was subsequently confirmed by Kopper and Steel (24) while studying colonic carcinoma and SCLC. The finding of a positive correlation between patient response and xenograft response in our study provides ample encouragement to researchers to pursue and develop preclinical assays, which are critical for the improvement of treatment of these cancers.

The key point is to understand why it is so difficult to cure SCLCs that are initially so chemosensitive. Resistance may occur at the initiation of therapy or may be acquired later. Multidrug resistance, which is developed in both SCLC and non-SCLC (10), may be responsible for the failure of chemotherapy. The present study was designed to test whether the MDR1 gene could be involved in the drug resistance of SCLC. MDR1 gene and P-glycoprotein expressions in SCLC remain controversial subjects (10,14). When activated, the MDR1 gene is expressed as a 4.5-kilobase (kb) mRNA in tumor cells selected for resistance to a panel of unrelated drugs and in some normal tissues (25-28). The expression of P-glycoprotein was shown to correlate with drug resistance in human tumors (29).

We evaluated the expression of MDR1 mRNA in our SCLCs and correlated this expression with the response of the xenografts and the clinical responses of the patient donors of the xenografts to combination chemotherapy. The MDR1 transcript was highly overexpressed in tumor SCLC-75, which originated from an untreated patient; SCLC-6 and SCLC-30, both of which also originated from untreated patients, expressed MDR1 to a moderate degree. Three of seven tumor lines (SCLC-10, SCLC-61, and SCLC-74A) from untreated patients had no detectable MDR1 mRNA levels. Two tumor xenografts (lines SCLC-41 and SCLC-74A) became positive for MDR1 mRNA expression after C'CAV chemotherapy in mice. In addition, no MDR1 gene mRNA transcript was found in the SCLC-74A tumor before treatment, but it was detectable in the SCLC-74B tumor that originated from the same patient after treatment with C'AV. These results suggest that the success of the chemotherapy is compromised when the drugs used select for MDR1 mRNA-positive cells. Similar observations have been previously reported in several other types of cancer (27,28).

The detection of MDR1 mRNA overexpression before treatment appears surprising, but cellular resistance to cytotoxic drugs can be acquired or intrinsic. Indeed, a neoplastic cell can be resistant to chemotherapy at the time of its malignant transformation or it can become resistant after exposure to therapeutic cytotoxic agents. Malignancies that exhibit de novo resistance are, by definition, refractory to chemotherapy and carry the worst prognosis. Many of the inherently resistant tumors may have been associated with increased exposure to mutagenic agents that may induce mutations in oncogenes. Indeed there is evidence that the MDR1 promoter is activated by mutated p53 and Ras proteins (30), as was reported in lung cancer and kidney cancer (31). SCLCs are chemosensitive tumors, and we observed a complete response to therapy in four of seven patients and in 26 of 62 nude mice with the corresponding xenografts. The efficiency of the four-drug combination chemotherapy was perhaps due to cisplatin and cyclophosphamide, both of which rarely select for the multidrug-resistant phenotype, although the MDR1 gene can be induced in human cells as a consequence of many types of cell damage (32). In our study, despite this initial positive response to C'CAV, the tumors regrew both in the patients and in the nude mice. This result suggests a multifaceted mechanism of resistance, including the resistance to alkylating agents.

GSTs are directly implicated in the protection of cells against cytotoxic and carcinogenic agents (33). The acidic class π protein was in general the most abundant GST form in all the human tumors studied so far (34). In our study, all tumors except SCLC-41 expressed detectable levels of this protein. Other mechanisms of resistance could account for the inability of chemotherapy to cure a majority of the SCLCs. Non-P-glycoprotein forms of multidrug resistance have been recently described (17). Using a resistant SCLC line, Cole et al. (17) discovered a new class of cell membrane proteins, designated multidrug-related proteins (MRP), structurally analogous to the P-glycoprotein. Such molecular determinants could play a role in this complex domain of multidrug resistance.

Histologically, there are two distinct forms of SCLC: 1) the oat-cell type consisting of homogeneous small cells and 2) the intermediate type recognizable by the heterogeneity of cell sizes. SCLC-10, SCLC-41, SCLC-61, and SCLC-74A tumors exhibited the oat-cell type. Two of these tumors (SCLC-10 and SCLC-61) came from patients who showed long-term survival. SCLC-74A evolved toward the intermediate-type histology of the SCLC-74B. Gazdar (14) suggested that the prognosis for patients with the intermediate type is worse than that for patients with the oat-cell type. In our limited series, we observed that the four lines that exhibited MDR1 gene expression (SCLC-74B, SCLC-75, SCLC-30, and SCLC-6) were of the intermediate type, and the survival of the patient donors of these lines was short. MDR1 expression could represent a marker of malignant evolution as described by Bradley et al. (35) and by us (36), just as the intermediate histologic type might be. However, because MDR1 imparts resistance in cells that express P-glycoprotein and since the prognosis of SCLC is closely related to the response to therapy, MDR1 expression may legitimately be considered prognostically significant, whatever its
true physiopathologic function is.

The key points of these studies are twofold: 1) The close correlation between results observed clinically and those obtained experimentally indicates that the human tumor transplanted into the nude mouse could be predictive of the results in the clinical setting; and 2) our data indicate that MDR1 gene activation may, indeed, be involved in the chemoresistance of SCLCs.

References


Notes

Affiliations of authors: M. F. Poupon, F. Arvelo, Y. Bourgeois, Unite de Recherche Associee 620 Centre National de la Recherche Scientifique, Institut Curie, Paris, France.
Present address: F. Arvelo, Centro de Biologia Celular, Facultad de Ciencias, Universidad Central de Venezuela, Caracas.
M. Jacrot, Laboratoire de Cytogetique, Faculte de Medecine, La Tranche, Grenoble, France.
Correspondence to: M. F. Poupon, M.D., Institut Curie, 26 rue d’Ulm, 75231 Paris cedex 05, France.
Supported by the Institut de la Santé et de la Recherche Medecale and the Association pour la Recherche sur le Cancer.
We thank Lorna Saint-Ange for reviewing the language used in this report.
Manuscript received April 14, 1993; revised August 26, 1993; accepted September 20, 1993.