Concordant Deletions of Chromosome 3p and Loss of Heterozygosity for Chromosomes 13 and 17 in Small Cell Lung Carcinoma

Naoki Mori, Jun Yokota, Mitsuo Oshimura, Webster K. Cavenee, Hideaki Mizoguchi, Masayuki Noguchi, Yukio Shimosato, Takashi Sugimura, and Masaaki Terada

National Cancer Center Research Institute, 1-1, Tsukiji 5-chome, Chuo-ku, Tokyo 104 [N. M., J. Y., M. N., Y. S., T. S., M. T.]; Department of Medicine, Tokyo Women's Medical College, 1, Kawada-cho 8-chome, Shinjuku-ku, Tokyo 162 [N. M., H. M.]; Kanagawa Cancer Center, Clinical Research Institute, Nakao-cho 54-2, Ashiku, Yokohama 241 [M. O.]; Japan; and Ludwig Institute for Cancer Research, Royal Victoria Hospital and McGill University Faculty of Medicine, Montreal H3A 1A1 [W. K. C.], Canada

ABSTRACT
Common regions of loss of heterozygosity on chromosomes 3, 13, and 17 were determined by restriction fragment length polymorphism analysis in 34 tumors and nine cell lines from 27 patients with small cell lung carcinoma. The common regions of loss of heterozygosity on chromosomes 3, 13, and 17 reside between D3S2 (3p14-p21) and ERBB2 (3p22-p24.1), between D13S1 (13q12) and D13S2 (13q22), and distal to MYH2 (17p13.1), respectively. Allele loss in each of these regions has been previously shown in several human tumors. Cytogenetic analysis was performed on three small cell lung carcinoma cell lines which had allelic loss on all three chromosomes, and although chromosome 3p deletions were observed in two of three cell lines, no obvious chromosomal abnormalities involving chromosomes 13 and 17 were detected. Mitotic recombination or mitotic nondisjunction rather than deletion may thus be the frequent chromosomal mechanism for attaining homozygosity of chromosomes 13 and 17 in small cell lung carcinoma.

INTRODUCTION
Several lines of evidence have suggested that inactivation of tumor suppressor genes (antioncogenes) by mutation or loss is implicated in the genesis of a variety of human tumors (1). Such loss may be effected by several chromosomal mechanisms, including mitotic nondisjunction with loss, mitotic nondisjunction with duplication, mitotic recombination, gene conversion, and deletion (2, 3). In fact, loss of genes at specific chromosomal loci does occur frequently in certain types of tumors as shown by cytogenetic or RFLP analysis (1, 4, 5). The regions of allelic loss detected by RFLP analysis are, in several tumors, concordant with the regions of chromosomal deletion detected by cytogenetic studies. However, in other tumors, RFLP analysis reveals aberrations not observed in cytogenetic studies. Such discrepancies likely occur because RFLP analysis is able to detect cytogenetically undetectable chromosomal changes, such as mitotic recombination and mitotic nondisjunction with duplication. Therefore, it is possible that tumor suppressor genes reside within the regions where loss of heterozygosity is commonly observed in tumors by RFLP analysis (6–8), even though no obvious chromosomal abnormalities were detected by cytogenetic studies. For this reason, it is highly important to understand the chromosomal mechanism of gene loss and to determine the common region of loss of heterozygosity in tumors by RFLP analysis using a number of polymorphic DNA markers.

In SCLC, a high incidence of chromosome 3p deletion was previously shown by cytogenetic studies (9, 10) and subsequently confirmed by RFLP analysis: loss of heterozygosity on 3p was observed in nearly 100% of SCLC (11–15). We have further demonstrated by RFLP analysis that loss of heterozygosity occurs not only on 3p but also on 13q and 17p in nearly 100% of SCLC (13), although no common cytogenetic abnormalities of chromosomes 13 and 17 have been reported. Thus, it was suggested that mitotic recombination and mitotic nondisjunction with duplication are frequent changes on chromosomes 13 and 17 in SCLC. In order to test this hypothesis and to determine whether a common region of loss of heterozygosity for these 3 chromosomes exists in SCLC, we performed RFLP analysis of chromosomes 3, 13, and 17 in tumors from 27 patients with SCLC using 17 polymorphic DNA markers. In 3 cases, cytogenetic characterization was also performed. Our results strongly suggest that deletion occurs frequently on 3p, and that mitotic recombination or nondisjunction is more frequent than deletion for 13q and 17p in SCLC. As a consequence of such chromosomal changes, loss of heterozygosity is commonly and simultaneously observed in nearly 100% of SCLC within 3 different chromosomal regions: 3p14-p24.1; 13q12-q22; and 17p13. It is of much interest that these 3 chromosomal regions show frequent loss of heterozygosity in various other types of tumors, suggesting that recessive genetic lesions in common chromosomal regions are involved in their development.

MATERIALS AND METHODS
Samples. Twelve primary tumors, 22 metastatic tumors, and 16 normal lung tissues were obtained from 22 patients with small cell lung carcinoma at surgery or autopsy. Corresponding normal tissues were available in 28 tumors from 16 patients (Cases 1 to 16), but were not available in 6 tumors from the other 6 patients (Cases 17 to 22). Nine SCLC cell lines were also used in this study. Three of 9 cell lines (Lu-135, Lu-140, and Lu-143), with both corresponding original tumors and normal tissues, were derived from 3 of the patients above (Cases 1, 2, and 6, respectively). One of 9 cell lines (Lu-139) with corresponding original tumor was derived from Case 17. No corresponding samples were available in the other 5 cell lines (Lu-24, Lu-130, Lu-134, Lu-138, and Lu-141). Primary tumors included both an untreated tumor (Case 11) and 4 of Stage 1 tumors (Cases 3, 6, 8, and 16). Histological diagnoses of tumors were made according to the Histological Typing of Lung Cancers defined by the WHO (16). Stages of tumors were determined according to the TNM Classification of Malignant Tumors defined by the International Union against Cancer (17). RFLP Analysis. High-molecular-weight DNA was prepared by proteinase K digestion and phenol/chloroform extraction. DNA (10 μg) was digested with appropriate restriction endonuclease, separated by agarose gel electrophoresis, transferred to nitrocellulose or nitroplus filters, and hybridized to 32P-labeled probes prepared by nick translation or random primer labeling. The blots were hybridized to the 17 poly-
morphic DNA probes homologous to loci on chromosomes 3, 13, and 17 listed in Table 1 (18-20). The allele lengths observed in all patients were identical to those published except for the RAGI locus as previously described (Footnote 4; Ref 19). Allele loss was considered to have occurred if its hybridization intensity was less than 10% of that in its corresponding normal tissue.

Cytogenetic Analysis. Three cell lines (Lu-135, Lu-140, and Lu-143) were cultured in RPMI 1640 supplemented with 10% fetal calf serum. For chromosome number, the conventional Giemsa staining method was used, and a total of 20 metaphases was examined. In each case, 15 metaphases were karyotyped by the quinacrine fluorescence banding method (21). The chromosome identification, including polymorphic patterns, was performed and described according to the nomenclature for an international system for human chromosomes (22).

RESULTS

RFLP Analysis of Chromosomes 3, 13, and 17 in 16 SCLC Patients. The DNA markers used for the analysis of chromosome 3 are the following: RAGI (3p24-p25); ERB18 (3p22-p24.1); DNF1SS2 (3p21); D3S2 (3p14-p21); D3S3 (3p14); and SST (3q28) (Table 1). Allelic deletions at one or more loci on chromosome 3p were observed in all 15 available patients (100%). Loss of heterozygosity was observed at all loci on 3p in 100% of the informative cases (1 of 1, 11 of 11, 2 of 2, and 8 of 8) at the RAGI, ERB18, DNF1SS2, and D3S2 loci, respectively. Constitutional homozygosity was observed in all 16 patients at the D3S3 locus. In contrast, heterozygosity at the SST locus (3q28) was retained in 7 of 7 tumors. In all 16 cases, loss of heterozygosity was observed at all informative loci within the RAGI, ERB18, DNF1SS2, and D3S2 group, while the SST locus retained heterozygosity when it was informative (Table 2). These results indicate that loss of heterozygosity usually occurs on the short arm but not on the long arm of chromosome 3 in SCLC, and that the common region of loss includes the sequences between D3S2 (3p14-p21) and RAGI (3p24-p25). 3p14-p25. The frequent chromosomal change on 3p appears to be large deletion or mitotic recombination rather than mitotic nondisjunction.

Allelic deletions of chromosome 13 were examined with 4 DNA markers: D13S1 (13q12); D13S2 (13q22); D13S4 (13q22); and D13S3 (13q33) (Table 1). Allelic deletions at one or more chromosomal loci were seen in 15 of 16 patients (93.8%). We previously reported the results of Cases 1 to 11 for SCLC and that the common region of allelic deletions lies between 13pter and 13q22, containing the RQ locus on 13q14 (13). In Case 14, the D13S1 locus showed allelic loss, but the D13S1 locus retained heterozygosity, suggesting that heterozygosity is lost between 13q12 and 13qter. In the other 4 cases, loss of heterozygosity was observed at all informative loci on chromosome 13 (Table 2). The common region of allelic deletion in 16 SCLC cases lies distal to D13S1 (13q12) and proximal to D13S2 (13q22): 13q12-q22. The possible chromosomal mechanisms effecting this included mitotic nondisjunction, mitotic recombination, and large deletion.

Polymorphic DNA markers for loci on chromosome 17 included the following: D17S30 (17p13); D17S28 (17p13); D17S1 (17p13); MYH2 (17p13.1); D17S71 (17p11-q11); GH2 (17q22-q24); and D17S4 (17q23-q25.3) (Table 1). All 16 patients showed loss of heterozygosity at more than 2 loci on chromosome 17. Loss of heterozygosity was observed at all loci on the short arm of chromosome 17 in 100% of informative patients (11 of 11, 3 of 3, 7 of 7, 10 of 10, and 10 of 10) at the D17S30, D17S28, D17S1, MYH2, and D17S71 loci, respectively. In contrast, loss of alleles at the GH2 locus on 17q was observed in only one of 12 patients. Constitutional heterozygosity was retained at the D17S4 locus on 17q in 2 of 2 informative patients. In all 16 cases, all informative loci between D17S71 and D17S30 showed loss of heterozygosity, while heterozygosity was retained at the GH2 and D17S4 loci except in Case 1, when they were informative (Table 2). These results indicate that the common region of allelic loss contains the part between D17S71 (17p11-q11) and D17S30 (17p13): 17p11-p13. Since allele loss rarely occurs on 17q, homozygosity appears to be effected by mitotic recombination or large deletion.

Loss of heterozygosity was detected not only in advanced stages of tumors but also in 4 cases of Stage I tumors (Cases 3, 6, 8, and 16 in Table 2). It was also observed in an untreated patient (Case 11). Primary tumors and metastatic tumors from the same patients always showed the same genotypes at all loci examined. Furthermore, the results showed no discrepancy between original tumors (Cases 1, 2, and 6) and corresponding cell lines (Lu-135, Lu-140, and Lu-143, respectively). A typical result of RFLP analysis of Case 6 and Lu-143 cell line is shown in Fig. 1. Loss of heterozygosity at loci on each of the 3 chromosomes was observed in 14 of 16 patients and at loci on 2 of the 3 chromosomes in the other 2 patients (Cases 3 and 8). In Case 3, constitutional homozygosity was observed at all 5 loci on 3p, so that information on 3p was not available. In Case 8, only one of 4 loci on 13q, D13S2, was informative, and heterozygosity was retained at this locus.

RFLP Analysis of Chromosomes 3, 13, and 17 in 12 SCLC Samples without Corresponding Normal Tissues. Because normal tissues from the same patients were not available in 6 tumors and 6 cell lines, we were not able to assess loss of heterozygosity by RFLP analysis. Nevertheless, since loss of heterozygosity on chromosomes 3p and 17p was observed in all informative patients, and that on 13q in nearly 100% of patients, it is highly possible that allelic deletions on these chromosomes have occurred in most of these tumors and cell lines, and that loci on each chromosome showing heterozygous genotypes in these tumors lie outside the common region. Therefore, genotypes in these samples were examined by the same method using the same DNA markers (Table 2).
Table 2  RFLP analysis of chromosomes 3, 13, and 17 in SCLC

The restriction fragment length alleles present in tumor at loci that were constitutionally heterozygous are indicated by 1 and 2: 1 indicates loss of the smaller sized allele; 2 indicates loss of the larger sized allele; 1,2 indicates that heterozygosity was retained in tumor; – indicates not informative; and absence of an entry indicates no data. Corresponding normal tissues were available in Cases 1 to 16, but not in Cases 17 to 22, Lu-24 to Lu-141. Lu-135, Lu-139, Lu-140, and Lu-143 were derived from Cases 1, 17, 2, and 6, respectively.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>–</td>
<td>–</td>
<td>2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1,2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>2</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1,2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>2</td>
<td>2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1</td>
<td>2</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>2</td>
<td>–</td>
<td>–</td>
<td>1</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>1,2</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1,2</td>
<td>2</td>
<td>1,2</td>
<td>–</td>
<td>–</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1,2</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>2</td>
<td>–</td>
<td>1,2</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>–</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>2,1,2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>2</td>
<td>1</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>1,2</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1,2</td>
<td>–</td>
<td>1,2</td>
<td>–</td>
<td>–</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>1,2</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>2</td>
<td>–</td>
<td>1,2</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1,2</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>1,2</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>2</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>2,2</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1,2</td>
<td>2</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>1,2</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1,2</td>
<td>2</td>
<td>–</td>
<td>2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1,2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>2,2</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1,2</td>
<td>1</td>
<td>–</td>
<td>1</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1,2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1,2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>–</td>
<td>–</td>
<td>1,2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>2,1,2</td>
<td>1</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>1,2</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1,2</td>
<td>–</td>
<td>–</td>
<td>1,2</td>
<td>–</td>
<td>–</td>
<td>1,2</td>
<td>–</td>
<td>–</td>
<td>1,2</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1,2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1,2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1,2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1,2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Lu-24</td>
<td>–</td>
<td>–</td>
<td>1,2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1,2</td>
<td>1,2</td>
<td>–</td>
<td>1,2</td>
<td>1,2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1,2</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Lu-130</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1,2</td>
<td>–</td>
<td>1,2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1,2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Lu-134</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Lu-138</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1,2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1,2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Lu-141</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1,2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
</tbody>
</table>
GENE LOSS ON CHROMOSOMES 3p, 13q, AND 17p IN SCLC

D13S2
MapI
C T N
-2.9

D13S1
MapI
C T N
-1.3

D17S30
TaqI
C T N
-3.0

D17S28
MapI
C T N
-2.4

SST
EcoRI
C T N
-12

D13S2
MapI
C T N
-15

GH2
MapI
C T N
-4.3

Fig. 1. Southern blot analysis of DNA from cell line (Lanes C, Lu-143), primary tumor (Lanes T), and normal tissue (Lanes N) from Case 6. Loci and restriction endonucleases used are shown above. Size of each polymorphic band in kilobases (kilobase pairs) is shown on the right.

The ERBAβ locus showed a heterozygous genotype in Case 17 and Lu-139 (derived from Case 17). Heterozygosity was observed at the SST locus in Cases 19, 20, and Lu-24. The other loci did not show a heterozygous genotype in all samples. In combination with the results of the 16 cases described above and of previous cytogenetic studies (3p14-p23 deletion) (9), the common region of loss of heterozygosity could lie between D3S2 and ERBAβ on the short arm of chromosome 3: 3p14-p24.1.

Case 19 showed heterozygous genotypes at the D13S4 and D13S3 loci. Case 21, Lu-24, and Lu-138 showed heterozygous genotypes at the D13S2, D13S3, and D13S4 loci, respectively. Heterozygosity was not observed at the D13S1 locus. Thus, the region between D13S2 and D13S3 (13q22-q33) may be outside the common region of allele loss on 13q. These results are consistent with that from RFLP analysis of the paired samples from the aforementioned 16 patients, suggesting the common region of allelic loss on 13q lies between 13q12-q22.

In Case 19, the MYH2 and GH2 loci showed heterozygosity while the other loci on chromosome 17 did not show heterozygosity. In Lu-24, heterozygosity at the D17S71, MYH2, and D17S30 loci was retained, but the D17S1 and D17S28 loci were not heterozygous. In contrast, the other 5 tumors and 5 cell lines did not show heterozygous genotypes at all loci on chromosome 17p, but all showed a heterozygous genotype at the GH2 locus. The D17S4 locus was heterozygous in Cases 18, 22, and Lu-138. The probabilities of constitutional homozygosity at D17S30, D17S28 and D17S1 in our 16 patients are P1 = 0.27 (4 of 15), P2 = 0.8 (12 of 15), and P3 = 0.56 (9 of 16), respectively. If the P1, P2, and P3 do not affect one another and we can apply the P1 to P3 to 6 SCLC cell lines and 6 tumors, the probability that Lu-24 and/or Case 19 indicates loss of heterozygosity on at least one locus within D17S30, D17S28, and D17S1 is 0.946 [1 - (P1 × P2 × P2 × P3 × P3)]. When corresponding normal tissues become available, it is likely that loss of heterozygosity at loci on 17p13 will be detected in Lu-24 or Case 19. In combination with the results of the 16 cases, the common region of loss of heterozygosity probably maps distal to MYH2 (17p13).

Comparison of RFLP and Cytogenetic Analyses in 3 SCLC Cell Lines. The results are summarized in Table 3. The modal chromosomal number of Lu-135 was 57 with a range of 55 to 59. Six to 8 marker chromosomes of unknown origin were observed in addition to the deletion of the short arm of chromosome 3 (probable break at Band p12). The 3p— consists of the long arm of chromosome 3 and unknown segments, and no heteropolymorphic patterns (22) were observed on chromosomes 3 and 13. The modal chromosomal number of Lu-140 was 72 with a range of 66 to 75. Ten to 14 marker chromosomes of unknown origin were observed, and no heteropolymorphisms for chromosomes 3 and 13 were apparent. The modal chromosomal number of Lu-143 was 61 (58 to 63) with 15 to 18 marker chromosomes. Deletion of the short arm of chromosome 3 (break at Bands p11-12) and heteropolymorphic patterns for chromosomes 3 and 13 were observed (Fig. 2). The 3p— consists of the long arm of chromosome 3 and unknown segments. The 2 apparently normal chromosomes 3 could be distinguished from the chromosome 3 carrying the deletion 3p— by the heteropolymorphic patterns. This result indicates that one of the apparently normal chromosomes 3 is in fact derived from another apparently normal chromosome 3 which may carry the mutation on 3p. Q-banding polymorphism observed on chromosome 13 also suggests that one of chromosome 13 is paternal and another is maternal at least on their centromeric region. No intact chromosome 17 was observed in Lu-143.

Deletions of the short arm of chromosome 3 were observed in Lu-135 and Lu-143, while Lu-140 showed no apparent structural abnormality involving chromosome 3. Loss of heterozygosity on 3p was observed in all 3 cell lines, constitutional heterozygosity was retained on 3q in Lu-135 and Lu-143, but definitive conclusions about 3q in Lu-140 could not be drawn. The results of the RFLP and cytogenetic analyses are consistent, and both indicate large deletions of chromosome 3p in Lu-135 and Lu-143, while mitotic recombination or mitotic non-

Table 3 Cytogenetic analysis and RFLP analysis of chromosomes 3, 13, and 17 in 3 SCLC cell lines

<table>
<thead>
<tr>
<th></th>
<th>Lu-135</th>
<th>Lu-140</th>
<th>Lu-143</th>
</tr>
</thead>
<tbody>
<tr>
<td>Modal chromosomal no.</td>
<td>57</td>
<td>72</td>
<td>61</td>
</tr>
<tr>
<td>No. of marker chromosomes of unknown origin</td>
<td>6-8</td>
<td>10-14</td>
<td>15-18</td>
</tr>
<tr>
<td>Chromosome 3 cytogenetic analysis</td>
<td>1/3p-</td>
<td>2-3</td>
<td>2/3p-*</td>
</tr>
<tr>
<td>RFLP analysis 3p</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>RFLP analysis 3q</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Chromosome 13 cytogenetic analysis</td>
<td>2</td>
<td>1-2</td>
<td>2*</td>
</tr>
<tr>
<td>RFLP analysis 13q</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Chromosome 17 cytogenetic analysis</td>
<td>2-3</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>RFLP analysis 17p</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>RFLP analysis 17q</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

* Q-banding heteropolymorphic patterns were observed on chromosomes 3 and 13 in Lu-143.
disjunction with duplication likely occurs in Lu-140. In these cell lines, although no obvious structural abnormality was observed involving chromosome 13, loss of heterozygosity on 13q was a common genetic alteration. In Lu-135 and Lu-140, mitotic recombination or mitotic nondisjunction with duplication may have occurred. It is supported by the heteromorphic patterns that mitotic recombination occurred in Lu-135. Lu-135 and Lu-140 showed no apparent structural change involving chromosome 17, and no chromosome 17 was identified in Lu-143, while RFLP analysis revealed that loss of heterozygosity had occurred on 17p in these cell lines. Because loss of heterozygosity was also observed on 17q in Lu-135, mitotic nondisjunction with duplication likely accomplished homozygosity, and since heterozygosity was retained on 17q in Lu-140 and Lu-143, mitotic recombination on chromosome 17 was possible. It is unlikely that there were submicroscopic small deletions in the regions without obvious structural change, since the regions of loss of heterozygosity on these chromosomes seem to be large enough so that such deletions could be microscopically detected.

**DISCUSSION**

In the present study, simultaneous loss of heterozygosity for loci on 3 different chromosomes was detected in nearly 100% of SCLC patients. The common regions of allele losses were 3p14-p24.1, 13q12-q22, and 17p13. Since loss of heterozygosity was detected in 4 cases of Stage I tumors as well as in an untreated tumor, it seems reasonable to propose that these were early genetic alterations in the development of the SCLC tumors.

Because loss of heterozygosity was not detected for loci on 3q (0 of 7) and was also rare for loci on 17q (1 of 12) by RFLP analysis, mitotic nondisjunction with loss or duplication was clearly not occurring frequently for these chromosomes. Rather, mitotic recombinational events seem a more likely alternative in concert with chromosomal deletion. In comparison with cytogenetic studies, RFLP analysis also indicated that mitotic recombination is the most frequent change on chromosome 17, and mitotic recombination or mitotic nondisjunction with duplication or loss may be more frequent than deletion on chromosome 13, while deletion occurs frequently on chromosome 3p in SCLC.

On chromosome 3p, the overlapping region of allelic deletion by RFLP analysis (3p14-p24.1) corresponds well with conclusions based on previous cytogenetic studies (9, 10). Renal cell carcinomas have also shown allelic losses in this region (23, 24). The common region of loss of heterozygosity for chromosome 13q in SCLC contains the **RB** locus, reminiscent of retinoblastoma (25). We and others previously described abnormal transcripts and the absence of **RB** protein in SCLC (26, 27), suggesting that the remaining allele of the **RB** gene is inactivated in these tumors as well as in retinoblastoma (28). Similar abnormalities of the **RB** gene have also been observed in osteosarcoma and breast carcinoma (28–30), in which the frequent occurrence of loss of heterozygosity on 13q has also been reported (31–33). These findings strongly suggest that complete inactivation of the **RB** gene is frequently the net result of loss of heterozygosity on chromosome 13q in several types of tumors. Though previous cytogenetic studies of SCLC showed no unique abnormality of chromosome 17 (9, 10), we have detected frequent losses of heterozygosity. Such alterations occurred at more than two loci in all 16 patients, and 9 of the 16 patients showed allelic deletions for virtually the entire short arm of chromosome 17. In combination with RFLP analysis of the 16 cases and studies of the 12 SCLC samples without corresponding normal tissues, the common region of allelic deletion was determined to reside distal to the **MYH2** locus on the short arm of chromosome 17. It is interesting that this region overlaps with that common alteration in colorectal carcinoma (proximal to **D17S30** on chromosome 17p) (8), osteosarcoma, and breast carcinoma (33, 34). It is possible that the same locus located in the common region of allele loss may be involved in the development of these different types of tumors. On the other hand, it is also possible that there may be lesions in more than two different genes that are differentially involved in each type of tumor and that map within the similar chromosomal regions. In either case, the present demonstration of concordant and simultaneous aberrations of 3 different chromosomal regions strongly supports the notion that SCLC is a progressive disease. Determination of the order of occurrence of the genetic lesions described here in the pathway of SCLC tumorigenesis should provide much useful information for understanding the basic biology and etiology of this disease.

**ACKNOWLEDGMENTS**

We thank the following scientists for providing DNA probes: Dr. G. Bell; Dr. R. White; Dr. J. Ghysdael; Dr. R. Evans; and Dr. W. E. C. Bradley. DNA probes were also obtained from the American Type Culture Collection, Rockville, MD, and from the Japanese Cancer Research Resources Bank, Tokyo, Japan.
REFERENCES


Concordant Deletions of Chromosome 3p and Loss of Heterozygosity for Chromosomes 13 and 17 in Small Cell Lung Carcinoma

Naoki Mori, Jun Yokota, Mitsuo Oshimura, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/49/18/5130

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.