Chromosome Alterations in Human Small Cell Lung Cancer: Frequent Involvement of 5q

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ABSTRACT

Deletions of the 3p chromosome region and molecular alterations of the tumor suppressor genes RB1 and TP53, located, respectively, at 13q14 and 17p13, are well-documented in small cell lung cancer (SCLC). Because of technical difficulties, karyotypes of primary SCLC specimens are rarely reported. In this study, detailed cytogenetic analysis was performed on 13 early passage SCLC cell lines and fresh specimens, including 4 lung primaries. Numerous chromosome alterations were found, even in newly diagnosed primary tumors. Consistent with previous molecular studies, chromosomal losses of 3p (13 cases) and 17p13 (12 cases) were frequently observed. Numerical losses of chromosome 13 and structural rearrangements affecting 13q14 were identified in 10 specimens. In addition, losses of chromosome 5 and structural alterations of 5q occurred in 12 tumors; among these, 9 displayed losses of region 5q13-q21. Double minutes were found in 4 cases (3 of 5 specimens from patients who received prior cytotoxic therapy but only 1 of 8 from untreated patients). DNA analysis revealed amplification of either MYC/J or MYCN in cells from each of these 4 tumors. Overall, the cytogenetic findings underscore that progression of SCLC involves multiple genetic changes and suggest further that a tumor suppressor gene(s) on 5q may contribute to SCLC tumorigenesis.

INTRODUCTION

In recent years, lung cancer has become the leading cause of cancer deaths among both men and women in the United States (1). SCLC (2) accounts for 20–25% of all lung cancer and is a distinct clinical and pathological entity with frequent paraneoplastic syndromes, almost invariable distant metastases, aggressive clinical course, and sensitivity to chemotherapy and radiation (2). Although combination chemotherapy can achieve objective responses in most cases of SCLC, 90–95% of patients die of their disease within 2 years (2).

In 1982, Whang-Peng et al. (3) reported a nonrandom chromosome abnormality, del(3p), in tumor cells from patients with SCLC. This cytogenetic alteration was identified in 16 of 16 cell lines and 4 of 4 fresh tumor specimens derived from bone marrow. In various cytogenetic investigations, the deleted region has been reported as 3p14-p23 (3, 4), 3p21-p22 (5), and 3p23-p24 (6). The frequency of 3p- in this neoplasm is controversial. Since the initial report of the 3p deletion in SCLC, this

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3 The abbreviations used are: SCLC, small cell lung cancer; RFLP, restriction fragment length polymorphism; FCS, fetal calf serum; HITES, RPMI 1640 medium supplemented with hydrocortisone (10 nm), insulin (5 µg/ml), transferrin (100 µg/ml), 17 β-estradiol (10 nm), and sodium selenite (30 nm); dmin, double minute chromosomes; ABR, abnormally banded region; HSR, homogeneously staining region.
All specimens and cell lines were processed for cytogenetics by overnight incubation in Colcemid (0.01 μg/ml), followed by treatment with 0.075 M KCl hypotonic solution for 30 min at 37°C and fixation in a 3:1 mixture of methanol:acetic acid. Chromosomes were analyzed using a G-banding technique. For each specimen, we attempted to obtain chromosome counts on at least 10 metaphase cells to determine the modal chromosome number. Whenever possible, karyotypes were prepared from at least 5 cells/case. Chromosome identification and karyotypic designations were in accordance with ISCN (1985) (21). In a given tumor, numerical changes were tabulated relative to the ploidy level of that specimen. For example, trisomy 7 in a near-triploid tumor is not considered to be a chromosome gain. An abnormal clone was defined as 2 or more cells with the same extra chromosome or structural anomaly or 3 or more cells with the same missing chromosome.

RFLP Analysis. DNA was extracted from newly established SCLC cell lines, fresh tumor tissues, and normal tissues. For each specimen, 10 μg of genomic DNA was digested to completion with restriction endonucleases, electrophoresed in 0.8% agarose gels, and transferred to a nylon support matrix (Gene Screen Plus; Dupont) by the method of Southern (22). The blots were hybridized overnight to a 32P random primer-labeled DNA probe at 42°C in 50% formamide, 1 m NaCl, 1% sodium dodecyl sulfate, and 10% dextran sulfate. DNA probes associated with RAF1 (3p25) consisted of a 5′ probe, which detects polymorphic alleles in EcoRI-cut DNA of 14.4 and 11.9 kilobases, and a 3′ probe, which detects polymorphic alleles in TaqI-cut DNA of 7.6 and 7.0 kilobases (23). The probe DNF1SS2 maps to 3p21 and detects polymorphic alleles in HindII-cut DNA of 2.3 and 2.0 kilobases (24). The probe D3S3 maps to 3p14 and detects polymorphic alleles in ApaI-cut DNA of 4.8, 3.6, and 1.2 kilobases (25, 26). DNF1SS2 was a gift of Dr. Ben Carritt, and D3S3 was kindly provided by Dr. Ray White. The probe D3S3 maps to 3p14 and detects polymorphic alleles in HindII-cut DNA of 7.6 and 7.0 kilobases (23). The probe DNF1SS2 maps to 3p21 and detects polymorphic alleles in HindII-cut DNA of 2.3 and 2.0 kilobases (24).

Assessment of Gene Amplification. DNA was isolated, and Southern blot analysis was performed as described above, except that the nylon membrane used was Hybond-C Extra (Amersham). The blots were hybridized overnight to a 32P random primer-labeled DNA probe at 42°C in 50% formamide, 5 m NaCl, 5% sodium dodecyl sulfate, 1% sodium dodecyl sulfate, 5% Denhardt's solution, and 10% dextran sulfate. Plasmid clone pNB-1 containing a 1.0-kilobase human MYCN fragment (27) and plasmid pE7 containing a 2.4-kilobase EGFR insert were obtained from the American Type Culture Collection repository. The clone pLMyc 10 containing a human MYCL fragment (28) was kindly provided by Dr. John Minna. The clone λ-LMC-12 containing a 1.5-kilobase CiaI-EcoRI MYC1 fragment (29) was a gift of Dr. Richard Hamelin. Amplification of genes was assessed by densitometric scanning of autoradiographs after normalizing to correct for slight loading differences between individual lanes of sample DNA.

RESULTS

Clinical findings from the 13 SCLC specimens and cell lines are summarized in Table 1, and cytogenetic findings are presented in Table 2. All 13 cases had complex karyotypes. Clonal abnormalities were identified in every case, but considerable karyotypic heterogeneity was observed in some specimens. The total number of numerical and structural changes ranged from 11–33/tumor (median number, 24). Seven to 22 structurally rearranged chromosomes were identified per specimen (median, 12).

The modal chromosome number was near-triploid in 8 cases, hyperdiploid in 1 case, near-diploid in 1 case, and hypodiploid in 3 cases. The incidence of near-triploid karyotypes among fresh tumor specimens (4 of 6 cases) was similar to that observed in cell lines (4 of 7). Near-triploidy was more common in specimens from untreated patients (6 of 8) than from treated patients (2 of 5). In contrast to this, hypodiploidy was observed in only one of the 8 untreated cases but was found in 2 of 5 specimens from treated patients. The overall number of structural and numerical alterations was very similar in fresh specimens and cell lines (median, 26 in fresh tumors versus 24 in cell lines) as well as among treated and untreated patients (median, 27 in untreated patients compared to 23 in treated ones).

All chromosomes contributed to numerical changes. Numerical losses were more frequent than gains. Losses of chromosomes 4, 5, 7, 10, 13, 15, 17, 21, 22, and the sex chromosomes were very common, each occurring as a consistent change in 7–11 specimens. The chromosomes most often involved in nu-

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Laboratory no.</th>
<th>Sex</th>
<th>Specimen (source)</th>
<th>Classification*</th>
<th>Prior treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>118 (B6T16)</td>
<td>M</td>
<td>Bone marrow*</td>
<td>Classic</td>
<td>Chemotherapy</td>
</tr>
<tr>
<td>2</td>
<td>008</td>
<td>F</td>
<td>Cell line (bone marrow)</td>
<td>Classic</td>
<td>Chemotherapy, chest radiotherapy</td>
</tr>
<tr>
<td>3</td>
<td>149 (UMC19)</td>
<td>M</td>
<td>Cell line (lymph node metastasis)</td>
<td>Variant</td>
<td>Chemotherapy, chest radiotherapy</td>
</tr>
<tr>
<td>4</td>
<td>051 (UMC22B)</td>
<td>M</td>
<td>Cell line (bone marrow metastasis)</td>
<td>Classic</td>
<td>Chemotherapy</td>
</tr>
<tr>
<td>5</td>
<td>065 (UMC25)</td>
<td>F</td>
<td>Cell line (periocardial effusion)</td>
<td>Classic</td>
<td>Chemotherapy</td>
</tr>
<tr>
<td>6</td>
<td>100 (UMC31)</td>
<td>M</td>
<td>Cell line (skin metastasis)</td>
<td>Classic</td>
<td>Chemotherapy</td>
</tr>
<tr>
<td>7</td>
<td>079,106,113 (UMC36)</td>
<td>F</td>
<td>Cell line (bone marrow)</td>
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<td>8</td>
<td>147 (UMC42)</td>
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<td>Cell line (lung primary)</td>
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</tr>
<tr>
<td>9</td>
<td>129</td>
<td>F</td>
<td>Tumor biopsy (lung primary)</td>
<td>ND†</td>
<td>Chemotherapy</td>
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<tr>
<td>10</td>
<td>160</td>
<td>M</td>
<td>Tumor biopsy (lung primary)</td>
<td>ND†</td>
<td>None</td>
</tr>
<tr>
<td>11</td>
<td>162</td>
<td>M</td>
<td>Tumor biopsy (lung primary)</td>
<td>ND†</td>
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<tr>
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<td>176</td>
<td>M</td>
<td>Tumor biopsy (lung primary)</td>
<td>ND†</td>
<td>None</td>
</tr>
<tr>
<td>13</td>
<td>209 (UMC45)</td>
<td>M</td>
<td>Tumor biopsy* (skin nodule)</td>
<td>ND†</td>
<td>None</td>
</tr>
</tbody>
</table>

* Classification of SCLC was based on presence or absence of l-dopa decarboxylase activity (18) and was performed only on established cell lines.
† Specimen later gave rise to a cell line.
‡ ND, not determined.
Numerical gains were numbers 1, 3, 7, 18, and 20, each identified in 4–6 cases. Among the structural rearrangements identified in this series, some chromosomes appeared to be frequently involved. In 12 cases, losses of chromosome 5 were found in all 13 specimens. Breakpoints involved in clonal alterations are shown in Table 1. Breakpoints clustered at 3p (11 cases, including those with polyomavirus-like infection of part or all of 17p: 7 specimens had derivatives with breakpoints or losses of chromosome 17. A gain of a chromosome 5 was observed in 12 specimens. Three (cases 1, 6, and 10) displayed numerical gains of chromosome 5, and 9 others had structural changes that resulted in partial losses of 5 (4 of the latter cases also were missing one chromosome 5). Three cases (2, 5, and 7) had an i(5p), 1 (case 4) had an interstitial deletion (5q11-q21), 2 (cases 9 and 12) had unbalanced translocation derivatives resulting in partial loss of 5q (shortest region of overlap, 5q31), and 3 cases (3, 11, and 13) had translocation derivatives containing part of 5q, with loss of 5pter-q13, 5pter-q14, or 5pter-q22, respectively. Gain of a chromosome 5 was observed in the remaining tumor (case 8). Loss of part or all of chromosome 17 was found in 12 of 13 specimens examined. In 4 cases, the only alteration of chromosome 17 was a numerical loss. Eight others had structural alterations that were interpreted as unbalanced rearrangements that would result in loss of part or all of 17p: 7 specimens had derivatives with breakpoints in either 17p (5 cases) or proximal 17q (2 cases), and 1 tumor had both a dicentric (15;17)(p12;p11.2) and a derivative chromosome. Examples of alterations of chromosomes 5 and 17 are shown in Fig. 3.

Rearrangements of chromosome 13 were less frequent, occurring in 5 cases. Three of these (cases 1, 11, and 13) had structural alterations with breakpoints at 13q42, and the other 2 had rearrangements that did not involve breakpoints or losses at this band. Numerical losses of chromosome 13 in the absence of structural change were observed in another 7 specimens.

Variable rearrangements of chromosome 1 occurred in 8 specimens. These included a 1p− (1 case), an i(1q) (1 case), and derivatives missing variable portions of 1p (6 cases); in 4 of 1324
these cases there were rearrangements of 1q, as well. Five translocation derivatives had breakpoints at 1p36.

Tumor cells from 4 cell lines (cases 1, 3, 4, and 6) had dmin. Three of these cell lines were derived from patients who had received prior cytotoxic therapy. In one of these lines (case 1), a minority of cells lacked dmin but, instead, had an altered chromosome 3 containing an ABR. In case 3, dmin were found in only a few cells, but a marker chromosome containing an HSR was present in nearly all cells examined. Among the 4 specimens with dmin, ABR, or HSR, 3 were considered to be cell lines at the time of cytogenetic analysis, and one eventually gave rise to a cell line. Southern blot analysis demonstrated amplification of either MYCI (cases 3 and 4) or MYCN (cases 1 and 6) in tumor cells from each of these 4 specimens (Fig. 2, B and C). Two cases exhibited a 5- to 10-fold level of amplification of MYCI (case 3) or MYCN (case 6). In each of the latter 2 cases, dmin were observed in a minority of the cells examined. The level of amplification of MYCN or MYCI was about 60-fold in cases 1 and 4, respectively. In each of these 2 cases, all of the metaphase cells examined contained dmin (or in case 1, an ABR in some cells), including a few mitoses with >100 of these minute bodies (Table 2). As controls, we also examined DNA from specimens not demonstrating cytogenetic evidence of gene amplification (cases 5, 7, and 8) using probes for MYCI, MYCN, MYCL, and EGRF; no evidence for amplification of any of the genes was found in these cases.

DISCUSSION

In this report, we describe detailed cytogenetic findings in 13 SCLC cases. Our data indicate that karyotypes in SCLC typi-
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Fig. 2. A, Southern blot analysis of HindIII-digested DNA from SCLC cell line UMC45 (case 13) and UMC-EBV45 (Epstein-Barr virus-transformed B-lymphoblastoid cell line from this same patient) hybridized with a radiolabeled DNF15S2 probe. Lane N, normal cells; lane T, tumor. Note loss of the 2.3-kilobase polymorphic allele in DNA from tumor cells. B and C, Southern hybridizations of EcoRI-digested DNA from 2 different blots showing amplification of specific MYC family genes in 2 SCLC cell lines. Lanes 1 and 2, DNA from SCLC cell lines with normal single-copy hybridization intensity; lane 3, DNA from SCLC lines exhibiting amplification of MYC1 or MYCN. B, approximately 60-fold amplification of MYC1 in case 4 (UMC22B); C, approximately 10-fold amplification of MYCN in case 6 (UMC31).

In addition to losses of 5q in our 9 cases summarized above, another tumor displayed an unbalanced translocation with loss of 5pter-q13. Thus, losses involving 5q13 were found in 10 SCLC cases overall. In addition, 2 near-triploid tumors had unbalanced derivatives, with loss of 5pter-q14 or 5pter-q22; in both cases, the derivative chromosome was accompanied by 3 apparently normal copies of chromosome 5. Thus, in these 2 cases, there is a net gain of part of the long arm of chromosome 5. A tumor suppressor gene at 5q13-q21 could be involved in these cases if, for example, nondisjunctional events have yielded 3 cytogenetically normal copies of the same homologue. In this model, each of these 3 homologues would harbor identical mutated tumor suppressor genes, while the other (normal) allele has been deleted from the unbalanced derivative chromosome. Alternatively, there may be a subset of SCLC tumors in which gain of part of 5q, rather than loss, is a significant change. Evidence in support of the latter proposal comes from case 8, in which the only change involving chromosome 5 was a numerical gain. However, as demonstrated for 3p alleles in case 13, loss of heterozygosity can exist in the absence of a structural change. Future RFLP analysis of this subset of SCLC tumors, using appropriate DNA markers for 5q, should resolve this issue.

In addition to losses of 5q in occasional SCLC tumors, gains of 5p can also occur. While each of our 3 cases with an i(5p) had a net loss of 5q, 2 of these tumors also displayed a net gain of 5p. Similarly, 3 of our SCLC cases had an i(3q), resulting in a net loss of 3p and a net gain of 3q. A major pathogenetic consequence of i(3q) and i(5p) may be the deletion of putative tumor suppressor genes on 3p and 5q, respectively. In addition, duplication of 3q or 5p by isochromosome formation could contribute to tumorigenesis by increasing the copy number of oncogenic sequences that reside on these chromosome arms.

The most consistent breakpoint in this series is at band 17p11 (Fig. 1). Overall, loss of all or part of 17p was seen in 12 of 13 (92%) cases. Morstyn et al. (14) also reported a high incidence of abnormalities of chromosome 17 in SCLC (8 of 10 cell lines), but the specific types of alterations and breakpoints were not described in detail. In another report, rearrangements of chromosome 17 were described in 4 of 11 SCLC specimens, including 3 cases with breakpoints at 17p11-p13 and apparent losses of part of 17p (13). In our series, structural changes included partial deletions and various derivative chromosomes with breakpoints in 17p or proximal 17q. In one study, loss of heterozygosity at 17p was demonstrated in 5 of 5 SCLC speci-

Fig. 3. G-banded chromosomes from several SCLC cases which exhibited alterations of both chromosomes 5 and 17. A, case 5: Note i(5p) and der(17)(17;17)(p11;?), B, case 12: der(5)(5;7)(q11;?) and der(17)(17;7)(p11;?); C, case 11: In addition to monosomy 17, there is a der(19)(5;19)(q14;q13.3 or q13.4). Arrows, rearranged chromosomes.
mensch (40). The tumor suppressor gene, TP53, is located at band 17p13.1 (41), and TP53 has been shown to be a frequent target for molecular alteration in lung cancer (42–44). The cytogenetic data presented here would appear to be compatible with the molecular evidence, if loss of 17p unveils a remaining, mutant TP53 allele on the other (karyotypically normal) homologue.

Overall, 10 of our specimens (77%) exhibited numerical losses of chromosome 13 or structural rearrangements affecting 13q14, site of the retinoblastoma susceptibility gene, RB1 (45–47). Absent or trace expression of the RBI tumor suppressor gene has been reported in nearly 80% of SCLC cases (48).

Rearrangements of 1p also were a rather frequent finding in this series. Eight cases showed rearrangements of 1p, including 5 with breakpoints at 1p36. Similar alterations of 1p have been reported by others (13, 14).

In our study, dmin, HSR, and ABR were seen in 4 of 13 (31%) specimens. Among 16 SCLC cell lines examined by Whang-Peng et al. (4), 2 exhibited dmin and one had an HSR. Wurster-Hill et al. (11) found dmin or HSR in 11 of 15 SCLC specimens from patients with late-stage disease and extensive distant metastases; 7 of the 15 specimens were obtained at autopsy. These novel cytogenetic alterations have been associated with amplification of oncogenes and genes involved in drug resistance (49, 50). Each of our cases with dmin, ABR, or HSR had amplified MYC1 or MYCN. In SCLC cell lines, amplification of a member of the MYC family of oncogenes is relatively common (28, 51, 52). However, in a study of primary tumors, amplification of MYC1 or MYCN was reported in only 5 of 45 specimens (53).

Cytological evidence for gene amplification was found in 3 of our 7 cell lines, but in only one of 6 fresh specimens. Among the 4 patients whose tumor cells contained dmin, ABR, or HSR, 3 received prior cytotoxic therapy. Overall, such cytogenetic alterations were identified in 3 of 5 specimens from previously treated patients versus only one of 8 from untreated patients. Likewise, in a recent report, amplification of one of the MYC family genes was detected in 28% of treated patient specimens, as compared to only 8% of the untreated patient specimens (54). This finding and the relatively low incidence of MYC family gene amplification in primary SCLC tumors (53) suggest that such amplification is unlikely to represent an initial, transformation-related event in SCLC.

In conclusion, the cytogenetic findings summarized here indicate that chromosome alterations in SCLC typically are very complex, even in newly diagnosed primary tumors. The abundance of cytogenetic alterations seen in these tumors implies that numerous genetic events are involved in the pathogenesis of SCLC. These data confirm that loss of 3p is a frequent occurrence in SCLC. Moreover, the recurrent losses of 17p and 13q documented cytogenetically in SCLC specimens are consistent with molecular data that demonstrate a crucial role of the tumor suppressor genes TP53 and RB1 located at these chromosome sites. In addition, our cytogenetic findings suggest that another tumor suppressor gene(s) located on 5q may also contribute to SCLC tumorigenesis, since losses at this region appear to be involved repeatedly in this malignancy. During disease progression, other acquired changes such as dmin, HSR, and abnormalities of 1p may also occur.

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