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 5q13-21. 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 MYC1 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 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 abnormality has been uniformly described in several other case reports (7–10). However, several investigators have reported a 3p- in only a minority of SCLC cell lines (11, 12) or fresh specimens (13). Morstyn et al. (14) reported structural alterations of 3p in 6 of 10 cell lines. These investigators concluded that there is not a unique chromosome abnormality present in all cases of SCLC, although loss of chromosome 13 and structural alterations of 3, 17, 1, 5, and 11 were common. However, RFLP analysis of 3p markers confirms an almost universal loss of genetic material in this region (15, 16).

Overall, the cytogenetic data base on SCLC is based largely on analyses of metastatic tumors, effusions, and established cell lines, and in many cases complete karyotypic information has not been presented. Because of technical difficulties, very few karyotypes of primary solid tissue specimens have been reported. In this study, we performed detailed cytogenetic analyses of tumor cells from 13 patients with SCLC. Included are findings from 6 fresh specimens and 7 new cell lines. Results of RFLP analyses and an assessment of gene amplification are also presented in selected cases.

MATERIALS AND METHODS

Chromosome analysis was performed on 6 fresh specimens (4 lung primaries, 1 metastatic skin nodule, 1 bone marrow aspirate) and 7 early passage cell lines from 9 male and 4 female patients. Two (cases 1 and 13) of the 6 fresh samples subsequently gave rise to cell lines; however, since the original cytogenetic analysis of these specimens was performed within 14–20 days of the sample acquisition date, these cases are grouped in this paper with other short-term cultures. Tissue specimens were obtained using institutional review board-approved protocols after obtaining informed consent from each subject or subject's guardian. Cytogenetic assessment of chromosome 3 and detailed RFLP analysis of 3p markers confirms an almost universal loss of genetic material in this region (15, 16).

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Short-Term Cultures. Solid tissue specimens were placed in a Petri dish, covered with 3–5 ml of complete medium, and cut into small pieces using scalpels. Both floating cell aggregates and sediment were washed twice with Hank's balanced salt solution and then distributed to several tissue culture flasks. Each specimen was cultured in RPMI 1640 medium supplemented with 10% FCS plus antibiotics and antimycotics. Metaphase cells were harvested for cytogenetics after 1–5 days of culture, except in case 13 which was harvested after 14 days of in vitro growth.

Establishment of Cell Lines. Specimens were prepared and placed in culture as described in detail elsewhere (17–19). For solid tumor specimens, single-cell suspensions were prepared from the primary or metastatic tumor tissues by mincing, passing through a stainless steel mesh, and gently pipetting with culture media. Bone marrow specimens were diluted to 10 ml with RPMI medium, and tumor cells were obtained by Ficoll-Paque (Pharmacia) centrifugation. The single-cell suspensions were approximately 1 × 10^6 cells/ml. Culture media consisted of (a) serum-free medium, HITES (20), (b) HITES and 5% FCS, and (c) McCoy's modified 5A, 10% FCS, and 20% heat-inactivated human erythrocyte fluid, as previously described (17). After the cell lines were well established (3–6 months), they were maintained in RPMI 1640 and 10% FCS. Biological and biochemical characterization of cell lines was performed as described elsewhere (17).

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This work was supported by NIH Grants CA-45745 and CA-06927 and by a grant from American Cancer Society and the Veterans Administration Research Service. The abbreviations used are: SCLC, small cell lung cancer; RFLP, restriction fragment length polymorphism; FCS, fetal calf serum; HITES, RPMI 1640 medium supplemented with 10% FCS plus antibiotics and antimycotics; ABR, abnormally banded region; HSR, homogeneously staining region.
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. 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 RAR1 (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 DNF1552 maps to 3p21 and detects polymorphic alleles in HinfII-cut DNA of 2.3 and 2.0 kilobases (24). The probe D3S3 maps to 3p14 and detects polymorphic alleles in MspI-cut DNA of 4.8, 3.6, and 1.2 kilobases (25, 26). DNF1552 was a gift of Dr. Ben Carritt, and D3S3 was kindly provided by Dr. Ray White. Blots were washed and autoradiographed as described previously (17).

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 μM standard sodium citrate (1 x solution is 0.15 M sodium chloride/0.015 M sodium citrate), 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 Clal-EcoRI MYCI 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, 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>
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</thead>
<tbody>
<tr>
<td>1</td>
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<td>Bone marrow</td>
<td>Classic</td>
<td>Chemotherapy, chest radiotherapy</td>
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<td>209 (UMC45)</td>
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<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 this group were chromosomes 1, 3, 5, 7, and 17, each of which were rearranged in at least 5 specimens. Breakpoints involved in clonal alterations are shown in Fig. 1. Breakpoints clustered at 3p (11 cases) including those with isochromosomes for 3q (resulting in duplication of the long arm or loss of the entire short arm), and 2 had both a deletion and either a derivative or an i(3q). The shortest region of overlap of losses of 3p is located at 3p21-p25. One tumor had both a dicentric (15;17)(p12;q11), along with variable non-clonal changes. Rearrangements of chromosome 13 were less frequent, occurring in 4–6 cases. Among these types of loss involving chromosome 5 were found in 12 specimens. Three (cases 1, 6, and 10) displayed numerical losses of chromosome 5, and 9 others had structural changes that resulted in partial losses of 5 (4 of the latter cases were also missing one chromosome 5). Three (cases 2, 5, and 7) had an i(5p), 1 case had an interstitial deletion (5q11-q21), 2 cases and 12 had unbalanced translocation derivatives resulting in partial loss of 5q (shortest region of overlap, q13-qter), 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 or gain of part 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 13q41, 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 i(1p) (1 case), an i(1q) (1 case), and derivatives missing variable portions of 1p (6 cases); in 4 of
CHROMOSOMES IN HUMAN SMALL CELL LUNG CANCER

In this report, we describe detailed cytogenetic findings in 13 SCLC cases. Our data indicate that karyotypes in SCLC typically are very complex, even in newly diagnosed primary tumors. While most of the current cytogenetic data base on SCLC has been obtained from cell lines, DeFusco et al. recently examined 11 fresh SCLC specimens (including 4 primary tumors) and found numerous chromosomal alterations in every case (13). Therefore, many cytogenetic changes appear to be involved in SCLC tumorigenesis, similar to data from nonsmall cell lung cancer (30, 31).

Cytogenetic evidence for loss of 3p was seen in 12 cases analyzed in our series. In one other specimen, the RFLP data demonstrated allelic losses at both the DNF15S2 (at 3p21) and 3'-RAFI (3p25) loci. Taken together, the cytogenetic findings and RFLP data indicate that all of our 13 SCLC cases are characterized by losses involving 3p. An interstitial deletion of 3p has been consistently identified in some studies (3, 4, 7–10, 19), but there are other reports in which only a minority of SCLC cell lines exhibited a deletion of 3p (11, 12, 14). In one study of fresh SCLC tumors grown in short-term culture, alterations of 3p were found in 5 of 11 cases examined (13). As we have noted elsewhere (17), some of the discrepancies among reports may be explained by differences in interpretation. Thus, whereas interstitial or terminal deletions of the short arm of chromosome 3 are not uniformly observed in SCLC, losses of 3p caused by other mechanisms have a comparable net result (32). For example, in our own series, 5 cases had deletions of 3p (accompanied by another altered chromosome 3 in 2 cases), 7 others showed losses of 3p due to unbalanced translocations or isochromosome formation, and 1 had (allelic) losses of 3p that were not evident from the cytogenetic analysis.

The shortest region of overlap of 3p losses among our cases is located at 3p21-p25, which overlaps with that reported in previous cytogenetic studies that placed the minimal deletion at 3p14-p23 (3, 4), 3p21-p22 (5), or 3p23-p24 (6). It is likely that some of these interlaboratory differences may be attributed to difficulties in precisely assigning breakpoints in some cases, particularly when the quality of the chromosome preparations is suboptimal, as is often the case in SCLC specimens. Unlike previous reports, our interpretation of the minimal 3p deletion extends more distally to 3p25 and is in agreement with molecular analyses demonstrating consistent loss of heterozygosity at the RAF1 protooncogene locus at band 3p25 in SCLC (17, 33, 34). Probes for anonymous sequences that detect RFLPs at 3p14 and 3p21 show that almost all SCLC tumors lose heterozygosity within these regions as well (5, 15, 16). Thus, the deleted segment in SCLC is typically large. This consistent loss of alleles at 3p in tumor tissue from essentially all patients with SCLC appears to strongly support the hypothesis that one or more putative tumor suppressor genes critical in SCLC tumorigenesis reside within this region.

Loss of 5q represents another frequent change in this investigation. In a review of karyotypic data presented in two previous reports (13, 14), losses of 5q were identified in approximately one-half of all SCLC specimens examined. Among our 9 cases with numerical losses of chromosome 5, interstitial deletions of 5q, i(5p), and various der(5) markers, the shortest region of overlap of deleted segments resides at 5q13-q21. This recurrent loss of 5q13-q21 suggests that this region may contain a tumor suppressor gene(s) important in the pathogenesis of SCLC. Interestingly, several cell regulatory genes are located within this region. One of these encodes the phosphatidylinositol-3 kinase associated protein, p85alpha, which has been mapped to 5q13 (35); this protein has been shown to modulate interactions between certain activated receptors and the phosphatidyl-
CHROMOSOMES IN HUMAN SMALL CELL LUNG CANCER

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).

inositol-3 kinase (36). The RASA locus which encodes the GTPase-activating protein involved in signal transduction and ZNF5 encoding a zinc finger protein have also been localized to 5q13 and 5q12-q13, respectively (cited in Refs. 37 and 38). It is also noteworthy that 2 genes (MCC and APC) located at 5q21 have been shown to be somatically altered in some colorectal tumors (39). It will be important to determine whether any of these genes is involved in SCLC.

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 gains 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. Mostyn 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;?)(p11;?) in addition to monosomy 17. B, case 12: der(5)(5;?)(q11;?) and der(17)(17;?)(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.
mensions (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 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, RBL (45–47). Absent or trace expression of the RBL tumor suppressor gene has been reported in nearly 80% of SCLC cases (48). Monosomy 13 or 13q deletions were observed in 6 of 10 SCLC cell lines examined by Morstyn et al. (14) and in 4 of 11 specimens analyzed by De Fusco et al. (13).

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 MYCl 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 MYCl 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 cytological 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 RBL 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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2. Naylor, S. L., Johnson, B. E., Minna, J. D., and Sakaguchi, A. Y. Loss of chromosome 17p 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).

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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 RBL 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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2. Naylor, S. L., Johnson, B. E., Minna, J. D., and Sakaguchi, A. Y. Loss of chromosome 17p 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 MYCl 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 MYCl 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 cytological 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 RBL 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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