Chromosome Abnormalities in Human Non-Small Cell Lung Cancer

Joseph R. Testa and Jill M. Siegfried

Abstract

Clonal cytogenetic abnormalities found in 30 non-small cell lung carcinomas (NSCLC), including 28 newly diagnosed primary tumor specimens, are summarized. Multiple chromosome alterations were identified in every case, and 19 of 30 tumors had near-triploid or near-tetraploid karyotypes. Polysomy 7 and partial gains of 7p, including 7p11–p13 (site of the EGFR gene), were particularly frequent, occurring alone or in combination in 26 tumors. Recurrent losses involving 1p, 3p, 6q, 9p, 11p, 15p, and 17p (where the TP53 gene is located) were each seen in 16–25 cases. Five tumors exhibited double minute, which were associated with amplified MYC1 (1 case) and EGFR (1 case), as determined by Southern analysis. The cytogenetic data were compiled from either short term cultures of tumor tissue harvested within 1–9 days (18 cases) or later harvests performed on long term cultures or cell lines (6 cases); in the other 6 cases results were obtained from both short term and long term cultures. Two studies were performed to validate the use of long term culture for cytogenetic analysis of solid lung tumors. First, in order to determine whether cytogenetic results from cultures are representative of the original tumor, the modal chromosome number of 13 specimens placed into culture was compared to the DNA index of the original tumor tissue, as measured by flow cytometry. The DNA indices of the solid tumor biopsies agreed with the degree of aneuploidy observed by cytogenetic analysis in every case. Second, in 6 cases we performed direct comparisons of karyotypes obtained from cells cultured by both methods. Identical chromosome abnormalities were detected in short term cultures and later harvests of the same specimen. Overall, our findings indicate that tumorigenesis in NSCLC is characterized by the accumulation of multiple chromosome alterations. Furthermore, these data demonstrate that recurrent cytogenetic changes can be identified in NSCLC and that detailed karyotypes from long term cultures are relevant to the original tumor. Chromosome abnormalities detected by these techniques may have clinical and biological significance. However, the complex pattern of karyotypic changes seen in newly diagnosed NSCLC emphasizes the need for future investigations of premalignant bronchial lesions in order to identify primary genetic changes important for early detection and intervention in this aggressive neoplasm.

Introduction

Cytogenetic analysis of lung tumors may reveal specific chromosome alterations that have clinical significance in prognosis and therapy. These clonal abnormalities may be indicative of critical molecular events in the etiology of lung carcinomas. NSCLC represent 75–80% of all lung tumors. Despite the high incidence of NSCLC, the cytogenetic data available are extremely limited in this neoplasm, compared with those for the less frequent hematological malignancies. Primary NSCLC specimens often have a low mitotic index, making it difficult to obtain adequate numbers of well banded metaphase cells for detailed cytogenetic analysis. Moreover, the karyotypes can be extremely complex, with many additional chromosomes, complicating efforts to identify consistent changes. Recently, several groups have reported detailed karyotypic findings in primary NSCLC (2–4); these studies have revealed multiple numerical and structural alterations in this neoplasm.

In this report, we summarize clonal cytogenetic changes found in 30 patients with NSCLC. We also validate the culture model used to produce actively dividing tumor cells, which provide better quality karyotypes. Although the karyotypic pattern appears to be very complex in NSCLC, several recurrent abnormalities have been identified, which should help to target specific chromosome sites for future molecular investigations. The data indicate that, as in colon cancer (5), tumorigenesis in NSCLC is characterized by the accumulation of multiple genetic alterations.

Materials and Methods

Cytogenetic analysis was performed on 28 newly diagnosed, fresh, primary NSCLC tumors and 2 pleural effusions. Findings in 21 of these patients have been described in detail in an earlier report (4).

Solid specimens were disaggregated mechanically. Hard specimens were also dissociated further by shaking the minced tumor pieces for 3–5 h in medium to which collagenase A was added, to a final concentration of 0.5 mg/ml. Following disaggregation, the cells were washed twice in Hanks' balanced salt solution, and then single cells and cell aggregates were suspended in complete medium and placed in culture. For 24 of these cases, the data presented were obtained from cultures for 9 days or less; of these 24, results were also obtained on six long term cultures (up to 80 days). In the other six cases, the cell lines that were established in culture were used for harvests at passage 3–20. Long term cultures were established using 3T3 feeder cell layers and conditioned medium from a lung carcinoma cell line, as described (6). Metaphase cells were arrested by exposing cells overnight to colcemid (0.01 μg/ml). Cells were detached from the surface of the glass using trypsin and a cell scraper, treated with 0.075 M KCl hypotonic solution for 30 min at 37°C, and then fixed in a 3:1 mixture of methanol:acetic acid.

Chromosomes were analyzed using G-banding or, occasionally, Q-banding techniques. For each specimen, we attempted to obtain chromosome counts on at least 20 metaphase cells to determine the modal chromosome number. Whenever possible, at least five karyotypes were cut out for each case.

Results

All 30 tumors exhibited complex karyotypes with many structural and numerical changes. While clonal abnormalities were present in all of these cases, every specimen showed considerable karyotypic heterogeneity. Aneuploidy was observed in all samples, with modal chromosome numbers clustering in the near-triploid to near-tetraploid range (19 specimens). Four cases had hypodiploid modal numbers, and five others were hyperdiploid, with 47–57 chromosomes. Two tumors had near-
All chromosomes contributed to numerical changes. The most frequent numerical alteration was polysomy 7, which was observed in 14 of 30 specimens (Table 1). In another six specimens there was partial trisomy of 7, with the shortest region of overlap of additional bands consisting of 7p11-p13. Six others had both polysomy 7 and +7p. Overall, gain of part or all of chromosome 7 was observed in 26 tumors; in 19 of these cases the number of 7/7p exceeded the number of copies expected based on the ploidy of the tumor. Polysomies of other chromosomes (e.g., 2, 11, and 20) were also prevalent, but their incidence did not appear to be remarkable when the ploidy of individual tumors was taken into account.

Among the structural rearrangements identified, some chromosomes tended to be involved repeatedly. Included in this group are chromosomes 1, 3, 6, 7, 8, 11, 13, 15, 17, and 19. Breakpoints in clonal rearrangements appeared to be distributed in a nonrandom manner (Fig. 1). The most frequently affected bands were 1p13, 3p13, 8p11-q11, 15p11-q11, and 17p11, each of which participated in rearrangements in 8–14 different specimens. There were no obvious differences among histological subtypes with regard to the particular kinds of rearrangements observed, except that isochromosomes for 8q (a clonal change in 6 cases) and 13q (two cases) and der(9q15q) (two cases) were seen only in adenocarcinomas. An isochromosome of 5p was identified in four tumors (two adenocarcinomas and two squamous cell tumors).

Balanced translocations seemed to be relatively rare in NSCLC. In contrast, chromosomal losses due to missing chromosomes or apparently unbalanced rearrangements (deletions and derivative chromosomes) were often observed (Table 2). In this series, there were recurrent losses of 1p, 3p, 6q, 9p, 11p, 15p, and 17p. Each of these losses was identified in at least 50% of all cases, with loss of 9p being the most frequent (25 of 30 cases). In some of these cases there was a 9p- or another unbalanced rearrangement affecting 9p, and in others the number of copies of chromosome 9 was less than that expected based on the ploidy of the specimen.

Tumor cells from five patients contained variable numbers of dmin. A sufficient amount of tumor specimen was available for molecular analysis in four cases, and evidence for gene amplification was found in two of these; one case had amplified MYC1, and the other had amplification of the EGFR gene.

To validate the culture system, the modal chromosome number of 13 cases was compared to the DNA index of the original solid tumor, as measured by flow cytometry. These were cases for which tissue was available for flow cytometry and a successful chromosome harvest was also obtained. Agreement between the predicted ploidy of the aneuploid or near-diploid peaks observed in the solid tumors and the modal numbers of cultures was found in all 13 cases (Table 3). Agreement was observed even in cases where the tumor population in the solid tissue was a minority of the cells in the flow cytometry analysis. In one case where two aneuploid peaks were detected by flow cytometry, a comparable wide range of chromosome numbers was found in culture.

Results from short and long term cultures were also compared in six cases, in order to determine how stable lung tumor karyotypes are in culture. The number of analyzable mitoses and the quality of chromosome banding typically were much higher in long term cultures than in short term harvests (Table 4). In addition, cytogenetic abnormalities were found to be virtually identical in cultures analyzed at different times. However, occasionally there was duplication of all chromosomes in some cells from long term cultures. This may mimic a "polyploidization" process which occurs in vivo to produce multiple, highly aneuploid populations.

Discussion

Our findings demonstrate that karyotypes in NSCLC generally are very complicated, even in newly diagnosed primary tumors. We have focused our investigations on samples obtained prior to initiating cytotoxic therapy; thus, the cytogenetic complexity seen in these cases appears to be part of the natural course of NSCLC. Furthermore, it is unlikely that this overall complicated pattern can be attributed to in vitro karyotypic evolution, because most of the analyses presented here are from relatively short term cultures of tumor cells. In most cases it was possible to obtain at least a few highly abnormal karyotypes from cells cultured for 9 days or less, and in approximately one half of all cases these data were from cells cultured for only 1–3 days. The agreement we found between modal chromosome number and DNA index further demonstrates that cells which proliferate in culture usually come from the major aneuploid or near-diploid component of the solid tumor. This suggests that our culture techniques allow us to draw valid conclusions about the chromosome abnormalities found in solid tumors.

Some lung tumor specimens were cultured on 3T3 feeder layers, from which we were able to obtain long term cultures.
In six of these cases we also harvested conventional short term cultures. In each of the latter cases the karyotypes obtained with the two methods were very similar, but the quality of chromosome preparations was superior with the feeder layer method. Thus, our preliminary studies suggest that the feeder layer method can be a useful procedure for the cytogenetic analysis of NSCLC specimens. Cell lines established by this method will also be useful for molecular analysis.

Polysomy 7 was a frequent numerical alteration, occurring in 20 of 30 (67%) specimens. Polysomy for all or part of chromosome 7 was identified in each of three lung adenocarcinomas examined by Jin et al. (2) and in all four lung adenocarcinoma cell lines reported by Fan and Li (7). Lee et al. (8) suggested that trisomy 7 is a very early change in NSCLC, and premalignant lesions for the presence of cells with early (primary) chromosome changes.

Table 2: Recurrent losses in 30 NSCLC cases

<table>
<thead>
<tr>
<th>Chromosome Arm</th>
<th>No. of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>3p</td>
<td>20</td>
</tr>
<tr>
<td>6q</td>
<td>15</td>
</tr>
<tr>
<td>9p</td>
<td>25</td>
</tr>
<tr>
<td>11p</td>
<td>17</td>
</tr>
<tr>
<td>15p</td>
<td>21</td>
</tr>
<tr>
<td>17p</td>
<td>21</td>
</tr>
</tbody>
</table>

*a* Losses due to missing chromosomes or apparently unbalanced rearrangements.

Table 3: Expected and experimentally determined modal chromosome numbers of primary lung tumors

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Expected modal number of tumor (approximate)</th>
<th>Modal number of tumor culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>±14</td>
<td>100/43</td>
</tr>
<tr>
<td>2</td>
<td>±14</td>
<td>100/43</td>
</tr>
<tr>
<td>3</td>
<td>±14</td>
<td>100/43</td>
</tr>
<tr>
<td>4</td>
<td>±14</td>
<td>100/43</td>
</tr>
<tr>
<td>5</td>
<td>±14</td>
<td>100/43</td>
</tr>
<tr>
<td>6</td>
<td>±14</td>
<td>100/43</td>
</tr>
<tr>
<td>7</td>
<td>±14</td>
<td>100/43</td>
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<tr>
<td>8</td>
<td>±14</td>
<td>100/43</td>
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<td>9</td>
<td>±14</td>
<td>100/43</td>
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<tr>
<td>10</td>
<td>±14</td>
<td>100/43</td>
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<tr>
<td>11</td>
<td>±14</td>
<td>100/43</td>
</tr>
<tr>
<td>12</td>
<td>±14</td>
<td>100/43</td>
</tr>
<tr>
<td>13</td>
<td>±14</td>
<td>100/43</td>
</tr>
</tbody>
</table>

*a* Based on DNA index of original solid tumor biopsy.

Polysomy 7 was a frequent numerical alteration, occurring in 20 of 30 (67%) specimens. Polysomy for all or part of chromosome 7 was identified in each of three lung adenocarcinomas examined by Jin et al. (2) and in all four lung adenocarcinoma cell lines reported by Fan and Li (7). Lee et al. (8) suggested that trisomy 7 is a very early change in NSCLC, and premalignant lesions for the presence of cells with early (primary) chromosome changes.

Loss of all or part of the short arm of chromosome 3 was identified in 20 (67%) cases in this series. Loss of heterozygosity for alleles on 3p has been reported in 25–100% of NSCLC cases in different reports (13–18). The shortest region of overlap of chromosome losses appears to be at 3p21. Allelic loss at 3p21 has been reported in all major types of lung cancer (14, 19). Deletion of 3p14–p23 was initially reported as a specific chromosome aberration in small cell lung cancer (20). More recently, deletions of 3p have been reported in a number of other malignancies, including renal cell carcinoma, mesothelioma, ovarian cancer, and breast cancer (21). Thus, loss of 3p may represent an important generalized tumorigenic event common to various solid tumors, including NSCLC.

As noted earlier, 9p was the most frequently lost chromosome segment in this series (25 of 30 cases). In a previous report, Lukeis et al. (3) identified deletions of 9p in 10 of NSCLC and suggested that this change may represent a critical event in this neoplasm. While only a minority of our cases had a 9p—

CHROMOSOMES IN NON-SMALL CELL LUNG CANCER

Table 4: Comparison of cytogenetic findings using two different culture methods

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Days in culture or passage number at harvest</th>
<th>Successful karyotypic analysis</th>
<th>Abnormal karyotype*</th>
<th>Abnormal cells (%)</th>
<th>Number of analyzable mitoses/slide*</th>
<th>Chromosome quality and banding quality</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9/9, 27</td>
<td>Yes/yes</td>
<td>Yes/yes</td>
<td>100/43</td>
<td>4.2/2.5</td>
<td>++/++</td>
</tr>
<tr>
<td>2</td>
<td>12/7</td>
<td>Yes/yes</td>
<td>Yes/yes</td>
<td>100/100</td>
<td>4.8/1</td>
<td>++/+</td>
</tr>
<tr>
<td>3</td>
<td>Passage 3/3</td>
<td>Yes/yes</td>
<td>Yes/yes</td>
<td>100/100</td>
<td>6.2/1</td>
<td>+++/+</td>
</tr>
<tr>
<td>4</td>
<td>Passage 4/5</td>
<td>Yes/yes</td>
<td>Yes/yes*</td>
<td>100/100</td>
<td>19/1</td>
<td>+++/+</td>
</tr>
<tr>
<td>5</td>
<td>80/1, 2</td>
<td>Yes/yes</td>
<td>Yes/yes*</td>
<td>100/100</td>
<td>23/1</td>
<td>+++/+</td>
</tr>
<tr>
<td>6</td>
<td>20/1, 2</td>
<td>Yes/yes</td>
<td>Yes/yes*</td>
<td>100/100</td>
<td>4.2/1</td>
<td>+++/+</td>
</tr>
</tbody>
</table>

*a* Karyotypic findings were similar in both types of cultures, unless indicated. , culture not successful.

Six to 10 slides were examined per case. We attempted to keep the density of nuclei equal for each case.

*a* Modal number and markers were similar in both cultures, but no completely analyzable metaphases were seen in short term culture.

*a* A few new changes were seen only in long term culture [i.e., +1, +del(3), +some cells with dmin]; also, unlike short term culture, the cell line exhibited a few polyploid cells (2 of 19). Breakpoint localization and interpretation of rearranged chromosomes were more precise using the cell line.

A few minor differences were seen between cultures (i.e., −15 seen only in short term culture; also −X and derivative marker were seen in several cells in short term culture but not long term culture). Polyploid cells were found in 3 of 9 cells in long term culture but in 0 of 6 cells from short term culture.
Fig. 2. Fluorescence in situ hybridization of a chromosome 7-specific, α-satellite probe to interphase nuclei and metaphase chromosomes (arrows) from normal diploid lymphocytes (A and B) and an aneuploid NSCLC cell line known from a previous karyotypic analysis to exhibit polysomy 7 (C and D). Note that lymphocytes have two fluorescent spots in each interphase nucleus and, correspondingly, two labeled chromosomes in the metaphase spread shown in B. The NSCLC cell line had three or four fluorescent spots in interphase nuclei (four in each nucleus shown here); three labeled metaphase chromosomes can be seen in the metaphase spread in D. The biotin-labeled probe is fluoresceinated and appears green under the microscope; the chromosomes are counterstained with the red-fluorescing DNA-specific dye propidium iodide.

others had either an apparently unbalanced rearrangement (various derivatives) affecting 9p or less than the expected number of copies of chromosome 9 (e.g., only two copies of chromosome 9 in a triploid tumor).

The pericentromeric regions of chromosomes 13–15 frequently participate in structural rearrangements in NSCLC. The 15p11-q11 region seems to be especially prone to cytogenetic change. All rearrangements affecting this site appeared to involve unbalanced derivative chromosomes which would result in loss of part of 15p. Thus, 15q11-qter could represent a site of a putative tumor suppressor genes or antioncogene (22). Among the abnormalities of chromosome 15 seen in this series was a der(9q15q), which was found in two adenocarcinomas and may represent a recurrent rearrangement in NSCLC. An apparently identical rearrangement was identified in a lung adenocarcinoma cell line reported by Fan and Li (7), and a der(15)(15;7)(p?;?) was identified in a lung adenocarcinoma examined by Jin et al. (2).

The most consistent breakpoint in this series was at band 17p11 (Fig. 1). Overall, rearrangements of 17p were seen in 21 (70%) cases. These structural changes include partial deletions and different derivative chromosomes involving 17p. Previous DNA analyses have demonstrated that loss of alleles on 17p often occurs in NSCLC (16). The TP53 tumor suppressor gene is located at band 17p13.1, and TP53 has been shown to be a frequent target for molecular alteration in lung cancer (23–25). Our data suggest that visible cytogenetic changes may be the cause of the allelic loss detected by molecular methods, at least in some cases. Taken together, the cytrogenetic and molecular evidence suggests that loss of 17p containing a normal TP53 allele unmasks a remaining mutant gene on the other (karyotypically normal) homologue.

While no balanced reciprocal rearrangements have been identified that are specific for a histological subtype of NSCLC, i(8q) was found only in patients with adenocarcinomas (a clonal change in six cases). Even though this isochromosome appears to be a recurrent finding in adenocarcinoma of the lung, i(8q) is not specific for this tumor. This abnormality has been reported in various types of leukemia and in several different solid tumors (1).

Variable numbers of dmin were seen in 5 of 30 (17%) specimens. It is now well known that these chromatin bodies are associated with amplification of oncogenes and genes involved in drug resistance (26, 27). We identified an amplified DNA
sequence in two of our cases with dmin; one case had amplified MYC1 and the other had amplified EGFR. In two series of NSCLC specimens, amplification of the MYC1 oncogene was documented in 3 of 36 (8%) and 4 of 25 (16%) cases (28, 29). Amplification of KRA S2 has also been reported in a case of NSCLC which contained dmin (30).

Overall, our cytogenetic findings indicate that chromosome alterations in NSCLC are often extremely complex, even in newly diagnosed primary tumors, but recurrent karyotypic changes can be found and these changes may have clinical and biological significance. The abundance of cytogenetic alterations identified in these tumors indicates that numerous genetic events can occur in NSCLC tumorigenesis. The recurrent loss of several specific chromosomal segments, including 3p, 9p, and 17p, provides additional evidence for a critical role of tumor suppressor genes at these sites in NSCLC. Other chromosomal imbalances, especially of chromosome 7, appear to have an important but as yet undetermined role in this neoplasm. The complex pattern of cytogenetic changes seen in newly diagnosed NSCLC emphasizes the need for future investigations of “normal” bronchus or premalignant lesions, in order to identify primary genetic changes important for early detection and intervention in this aggressive neoplasm.

Acknowledgments

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References

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