Loss of Heterozygosity at Chromosomes 3p and 17p in Primary Non-small Cell Lung Cancer

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Abstract. Background: Loss of heterozygosity (LOH) of selected regions at chromosomes 3p and 17p in non-small cell lung cancer (NSCLC) and the association of these abnormalities with major clinical parameters and prognosis were studied. Materials and Methods: The study group included 92 consecutive primary NSCLC tumours and four microsatellite markers from chromosome 3p and three markers from 17p were analyzed. Results: LOH of at least one locus was found in 83% of all analyzed tumours. Most frequently deletion (58%) was found at locus D3S1481 (3p14.2). Sequence deletions of D17S520 (17p12) and TP53 (17p13.1) occurred in 52% of tumours. LOH occurrence at 3p and 17p was more frequent in squamous cell carcinomas compared to adenocarcinomas (89% vs. 75%), but this difference was not significant. Conclusion: No significant association was found between LOH on any analyzed loci and tumour stage (TNM) and grade (G). There was no correlation between LOH and survival.

Non-small cell lung cancer (NSCLC) is characterized by multiple genetic changes frequently involving tumour suppressor genes (TSG) and oncogenes (1-5). The major genes whose loss of function occurs in the pathogenesis of lung cancer include: TP53 (chromosome 17p13.1), RB1 (13q), p16INK4a (9p21), FHIT (3p14.2) and RASSF1A (3p21.3) (1-4, 6). In NSCLC, allelic loss of heterozygosity (LOH) is frequently seen at certain chromosomal loci: 3p, 6q, 8p, 9p, 13q, 17p, 18q and 19q (3, 5-11). LOH on chromosomes 3p and 17p is the most frequent genetic alteration in lung cancer. At least five distinct regions of 3p are predisposed to homozygous deletions both in tumour samples and cell lines: 3p25-26, 3p24, 3p21.3, 3p14.2 and 3p12 (12-14). This suggests that at least one tumour suppressor gene, important in the pathogenesis of lung cancer, is localized in these chromosomal regions. Some authors suggested that LOH at 3p and 17p may be a crucial step in the early phase of tumorigenesis and that this alteration is retained throughout tumour progression (15, 16). Allelic deletions at some other loci, like 2q, 9p, 18q and 22q, accumulate during tumour progression (17).

Most studies have not demonstrated any apparent association between 3p LOH occurrence and major clinical characteristics (11, 14, 18). However, the prognostic value of LOH in NSCLC remains debatable (11, 14, 18-20). Some authors found shortened survival associated with LOH at 10q in primary tumour (11) or LOH at 3p and 5q in lymph node metastasis (14), but others did not demonstrate any prognostic implications of this abnormality (18-20).

The aim of this study was to analyze the occurrence of LOH of selected loci at chromosomes 3p and 17p in primary NSCLC, to assess the relationship between LOH and major clinical variables, and to determine the prognostic value of these alterations.

Materials and Methods

Patients. The study group included 92 consecutive NSCLC patients who underwent curative pulmonary resection between 1997 and 2000 (Table I). Histological diagnosis was performed according to WHO criteria. Pathological TNM stage was assigned according to the International Union Against Cancer (UICC) classification of 1997.

PCR and microsatellite analysis. DNA was extracted from frozen tumour fragments and blood samples obtained from the same
patients. Genomic DNA was isolated using a standard procedure with proteinase K digestion and phenol-chloroform extraction. LOH was determined by PCR-based polymorphic microsatellite markers specific for most common deletions in NSCLC. Four microsatellite markers from chromosome 3p: D3S1101 (3p12), D3S1481 (3p14.2), D3S643 (3p21.33) and D3S1435 (3p25), and three markers from chromosome 17p: D17S520 (17p12), TP53 (17p13.1) and 19B12 – 44% (28/63). This tendency was seen for LOH at 3p12, 3p25, 17p12 and 17p13.3 (45% vs. 31%, 49% vs. 27%, 61% vs. 51% vs. 31%, respectively; p=0.24).

Results

LOH of at least one locus was present in 83% of all analyzed tumours (Table II). Within the chromosome 3p, the samples were informative at the D3S1101, D3S1481, D3S643 and D3S1435 loci in 68%, 50%, 72% and 67% of cases, respectively. The most commonly affected locus at chromosome 3p was D3S1481 (58%; 26 out of 45 informative cases), followed by D3S1435 (43%; 23/54), D3S1101 (42%; 26/62) and D3S643 (40%; 19/47).

Within the chromosome 17p, heterozygous genotypes in DNA from blood samples were found in 82%, 61% and 83% of D17S520, p53 and 19B12 loci, respectively. The frequency of LOH on 17p was as follows: D17S520 - 52% (39 out of 75 informative cases), p53 - 52% (24/46) and 19B12 - 44% (28/63).

LOH on chromosome 3p or 17p occurred somewhat more frequently in squamous cell carcinomas (SCC) than in adenocarcinomas (AC) (89% vs. 75%, respectively; p=0.04). This tendency was seen for LOH at 3p12, 3p25, 17p12 and 17p13.3 (45% vs. 31%, 49% vs. 27%, 61% vs. 40% and 51% vs. 31%, respectively), however none of these differences was statistically significant. No relationship between LOH and other major clinical parameters, including tumour stage and differentiation, was found.

The median survival in the entire group was 30 months and the survival probability at 3 years was 45%. In univariate analysis, no relation between any LOH and overall survival was observed (informative vs. non-informative cases and LOH ratio <0.7 vs. LOH ratio >0.7, log-rank test). No survival difference was also found when the LOH ratio for each chromosome locus was entered into the univariate Cox proportional hazard model to check the impact of various LOH ratio cut-off points. No adjustment for multiple testing was applied.

Discussion

In this study, we searched for microsatellite deletions at chromosomes 3p and 17p, previously demonstrated as most frequently affected in NSCLC.
The frequency of 3p LOH of all markers in previously reported NSCLC series varied between 25 and 100% (11, 12, 17, 21-23). In our study, the most frequent deletion (58% tumours) was found at D3S1481 (3p14.2), whereas other loci from chromosome 3p were lost in 40-43% of cases. Other studies demonstrated LOH at 3p14.2 in 42-67% tumours (7, 11, 12, 23). Marker D3S1481 is localized in the third intron of the FHIT gene (23). FHIT is a TSG candidate and is often mutated in both small cell lung cancer (SCLC) and NSCLC (80% and 40%, respectively) (24). Garinis et al. showed that 71% of stage I NSCLCs displayed allelic loss in markers D3S1300 and D3S4103, both located in FHIT loci (25). The common occurrence of LOH in this region supports the notion that FHIT plays an important role in early lung carcinogenesis, although variable FHIT transcripts were also found in non-neoplastic tissues (2, 25, 26).

In this series, D3S643 (3p21.33) was deleted in 40% of all tumours. These sequences are localized 2 Mb upstream of the 630 kb region, which is often deleted in lung cancer and in other epithelial tumours. A total of 25 TSG candidate genes are localized in this region, of which the most frequently affected in lung cancers are CACNA2D2, BLU, RASSF1, FUS1, HYAL2, HYAL1, SEMA3F, RBM5 and RBM6 (27-30).

Several reports suggested that the 3p25-p26 region may contain TSGs, although no candidate gene was reported. Frequent LOH and the presence of homozygous deletions were reported in cancers of the lung, kidney and breast, and in other solid malignancies. In our study, D3S1435 (3p25) was lost in 43% of informative cases. These sequences are localized 100 kb upstream from the VHL gene (3p25.3) and their loss is associated with familial kidney cancer, multiple benign tumours of the retina, brain and pancreas. It is unlikely though that this molecular change is directly involved in lung carcinogenesis (27).

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The chromosome region 3p12-p13 was found to be frequently deleted or rearranged in lung cancer and in other malignancies. The marker D3S1101, used in our study, corresponds to the locus 3p12, which was lost in 43% of samples.

The TP53 gene, located at chromosome 17p13.1, is the most frequently mutated TSG in human malignancies. Mutations of this gene were detected in 21-75% of NSCLCs (4, 6, 14, 31, 32). In our group, LOH in the TP53 polymorphic marker was found in 52%, which is similar to the results obtained in other series where this abnormality occurred in 45-75% (1, 6, 14, 32). Konishi et al. demonstrated another commonly deleted region at 17p13.3, which is not related to either p53 mutations or 17p13.1 deletions (32). These findings, confirmed by other groups (15), suggest the existence of putative lung cancer TSG with possible localization at 17p13.3. In our study, the 19B12 marker from this chromosomal region was deleted in 44% of the analyzed samples.

The frequency of LOH in D17S520 (17p12) in our series (52%) was similar to that in TP53. Konishi et al. observed deletion of this marker in 21% of NSCLCs and in 92% of SCLCs, yet no TSG candidate related to lung carcinogenesis was reported in this chromosome region (32).

In our study, LOH on chromosome 3p or 17p occurred somewhat more frequently in SCC than in AC (89% vs. 75%), although this difference was not statistically significant. These results should be interpreted with caution though, due to the relatively small number of patients in both groups (63 SCC and 20 AC). However, a higher frequency of LOH in SCC than in AC is concordant with some previous studies (6, 7, 11, 32). There are important biological differences between these two histological subtypes, suggesting a distinct genetic background for their differentiation mechanisms and tumorigenesis (11, 33, 34).

### Table II. Overall frequency of LOH in particular loci in informative cases.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Chromosome band location</th>
<th>SCC n=63</th>
<th>AC n=20</th>
<th>LCC n=7</th>
<th>ASQC n=2</th>
<th>Total N=92</th>
</tr>
</thead>
<tbody>
<tr>
<td>D3S1101</td>
<td>3p12</td>
<td>20/44 (45)</td>
<td>4/13 (31)</td>
<td>2/3 (67)</td>
<td>0/2</td>
<td>26/62 (42)</td>
</tr>
<tr>
<td>D3S1481</td>
<td>3p14.2</td>
<td>19/33 (58)</td>
<td>6/8 (75)</td>
<td>1/3 (33)</td>
<td>0/1</td>
<td>26/45 (58)</td>
</tr>
<tr>
<td>D3S643</td>
<td>3p21.33</td>
<td>13/29 (45)</td>
<td>5/12 (42)</td>
<td>1/4 (25)</td>
<td>0/2</td>
<td>19/47 (40)</td>
</tr>
<tr>
<td>D3S1435</td>
<td>3p25</td>
<td>19/39 (49)</td>
<td>3/11 (27)</td>
<td>1/3 (33)</td>
<td>0/1</td>
<td>23/54 (43)</td>
</tr>
<tr>
<td>D17S20</td>
<td>17p12</td>
<td>28/46 (61)</td>
<td>8/20 (40)</td>
<td>2/7 (29)</td>
<td>1/2</td>
<td>39/75 (52)</td>
</tr>
<tr>
<td>TP53</td>
<td>17p13.1</td>
<td>16/29 (55)</td>
<td>6/11 (54)</td>
<td>2/4 (50)</td>
<td>0/2</td>
<td>24/46 (52)</td>
</tr>
<tr>
<td>19B12</td>
<td>17p13.3</td>
<td>21/41 (51)</td>
<td>5/16 (31)</td>
<td>2/5 (40)</td>
<td>0/1</td>
<td>28/63 (44)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>56/63 (89)</td>
<td>15/20 (75)</td>
<td>4/7 (57)</td>
<td>1/2</td>
<td>76/92 (83)</td>
</tr>
</tbody>
</table>

We did not find any significant association between LOH at 3p and 17p, and disease stage and grade. Of the three studies addressing this issue (7, 11, 12, 23), only one (12) demonstrated an association between LOH and tumour stage and grade, and this relation was confined to AC. Some authors found an association between the intensity of cigarette smoking and high frequency of LOH in 3p14.2 or 17p13.1 loci in NSCLC (6, 23). We did not analyze this relationship, as all patients were cigarette smokers.

In this series, no single LOH was associated with prognosis. Also, no prognostic impact of LOH on 3p and 17p in primary tumour was demonstrated in other series (11, 14, 18). However, in one of these studies, the presence of LOH on chromosome 3p in lymph node metastases indicated a worse survival (14). In another study, LOH at 3p was associated with shorter survival in AC but not in SCC (7).

During tumour progression, lung cancer cells accumulate many genetic changes and the assessment of their clinical implications is difficult. Therefore, more precise techniques and properly selected material are necessary to create an optimal system for the combination of molecular and clinical data for prognostic purposes.

Acknowledgements

Supported by the State Committee for Scientific Research, grant number: 4P05C04112 and partially supported by a grant from the Leopold Kronenberg Foundation. Karolina Ochman is the recipient of a scholarship from the Postgraduate School of Molecular Medicine, affiliated with the Medical University of Warsaw, Poland.

References


