Microsatellite Alterations in Bronchial and Sputum Specimens of Lung Cancer Patients

Monica Miozzo, Gabriella Sozzi, Katia Musso, Silvana Pilotti, Matteo Incarbone, Ugo Pastorino, and Marco A. Pierotti

Abstract

Early diagnosis of lung cancer based on conventional screening procedures has been unable thus far to decrease lung cancer mortality. We explored the possibility of using microsatellite instability in the detection and screening of early phases of lung carcinogenesis. We studied tumor, histopathologically normal bronchial mucosa, and cytological specimens of 51 lung cancer patients for the presence of clonal variations at microsatellite polymorphisms. Microsatellite alterations were found in tumor, normal bronchial mucosa and cytological specimens of 25 of 51 (49%) of the patients. The detection of microsatellite alterations in histopathologically normal bronchial specimens and cytological clinical samples with minimal atypia suggests a possible application of this genetic marker in early diagnosis of precancerous lesions and lung cancer.

Introduction

Instability at widespread highly polymorphic tandem repeat DNA sequences, known as microsatellites, has been reported in hereditary nonpolyposis colorectal cancers patients (1) and has been recently extended to other sporadic and familial cancers including colon, breast, and lung (2-4). The extension and the type of microsatellite alterations in sporadic tumors is generally less pronounced than in hereditary nonpolyposis colorectal cancer patients and often appears as one or few additional alleles at only one or few loci (5). In lung cancer, expansion or contraction of these repeat elements thus far has been reported in invasive tumors including 50% of SCLC (6) and in a fraction, ranging from 7 to 34%, of non-small cell lung cancer (4, 6-8). In addition, only one study describes the finding of microsatellite alterations in surgical resection margins of two non-small cell lung cancers and in the sputum of two SCLC patients (6). To determine if microsatellite instability satisfies the requirements for a suitable diagnostic marker in lung carcinogenesis, i.e., high frequency of alterations and early occurrence, we have examined 51 lung cancer patients for the presence of microsatellite instability, not only in their tumor tissue but also in histopathologically normal bronchial mucosa sampled at different sites of the bronchial tree away from the resected tumor. The ability to detect simple and early clonal genetic changes in these bronchial specimens would indeed constitute a valuable help for early detection of precancerous lesions and lung cancer development. In addition, we evaluated the corresponding sputum samples of the patients whose surgical tissue was positive for microsatellite alterations to test the possibility of using microsatellite instability detection in noninvasive routine clinical practice. Correlation between microsatellite instability and p53 overexpression has been also examined in tumor specimens.

Materials and Methods

Fifty-three tumors and 42 bronchial mucosa samples were obtained from 51 surgically treated lung cancer patients at Istituto Nazionale Tumori (Milan, Italy). They included 25 cases of squamous cell carcinoma, 18 adenocarcinoma, 5 SCLC, and 5 cases of other histotype (Table 1). The bronchial mucosa specimens were subjected to histopathological examinations. In addition, bronchial specimens were obtained from six control individuals undergoing surgical resection for nonneoplastic processes or for lung metastases of tumors out of the aerodigestive field. The bronchial mucosa samples were taken in a tumor-free area.

In all cases, patients' fresh blood was collected and used as source for normal DNA. DNAs from frozen tissues and peripheral blood lymphocytes were extracted using standard methods (9). The cytological material was mechanically scraped from the glass slides by using ethanol, and DNA was extracted using standard methods (9). The cytological material was extracted using standard methods (9). The cytological material was extracted using standard methods (9). The cytological material was extracted using standard methods (9).

PCR amplifications were performed in a volume of 50 μl containing 50-100 ng of DNA, 0.2 μM of each primer, 200 μM of each deoxynucleotide triphosphates, 1.5 mm MgCl₂, 5 μl PCR buffer (Perkin-Elmer), and 1.5 units Taq polymerase (Perkin-Elmer). Thirty-five cycles of PCR were carried out consisting of 30 s at 94°C, 30 s at annealing temperature (ranging from 50°C to 58°C), 30 s at 72°C, using a Perkin-Elmer PCR Thermocycler system. The PCR products were resolved on ethidium bromide-stained, non-denaturing 6.0% polyacrylamide gels according to the method described (14). Immunocytochemical staining of the p53 protein on frozen section was carried out following procedures reported previously (15).

Results

We tested 53 tumor and normal DNAs from 51 patients for the presence of microsatellite instability using di-, tri-, and tetranucleotide repeat markers listed in Table 2. In 42 of the patients, we could examine bronchial specimens taken far away from the tumor in the resected surgical sample. The average distance from the neoplasia ranged from 1 to several inches, depending on the extent of the surgical resection the patient underwent. One cytological slide of preoperative sputum sample was also available in five patients. The clinical and pathological features are reported in Table 1. DNAs extracted from blood, tissues, and cytological specimens were subjected to PCR amplification at four microsatellite loci in several (two to five) independent experiments.

Microsatellite Alterations in Tumor and Distant Bronchial Mucosa Specimens. Overall, 25 of 51 patients (49%) revealed the presence of one or two novel bands, absent in the paired normal DNA (Table 2). In 21 patients, these alterations involved a single locus, whereas in four they were observed at two loci. In particular, microsatellite instability was detected in 17 of 53 (32%) tumor DNAs
Table 1
Clinicopathological features of the patients* and tumor p53 staining

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>No.</th>
<th>%</th>
<th>p53 staining</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>SCC**</td>
<td>25</td>
<td>48</td>
<td>22**</td>
</tr>
<tr>
<td>ADC</td>
<td>18</td>
<td>34</td>
<td>16**</td>
</tr>
<tr>
<td>SCLC</td>
<td>5</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>Other</td>
<td>5</td>
<td>9</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 2
Frequency of microsatellite alterations

<table>
<thead>
<tr>
<th>Microsatellite polymorphism</th>
<th>Patients*</th>
<th>Tumor*</th>
<th>Bronchus*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>AR (Xq11) (GAG)19(CAA)</td>
<td>15/51</td>
<td>29</td>
<td>8/52</td>
</tr>
<tr>
<td>D3S1340 (3p24) (CA)9</td>
<td>3/34</td>
<td>9</td>
<td>3/34</td>
</tr>
<tr>
<td>D3S1339 (3p21) (CA)9</td>
<td>1/37</td>
<td>3</td>
<td>0/36</td>
</tr>
<tr>
<td>Total</td>
<td>15/51</td>
<td>29</td>
<td>8/52</td>
</tr>
</tbody>
</table>

* Number of patients displaying, in at least one of the tissues examined (tumor or bronchus), alterations of the marker.
** Number of tumor specimens with alterations of the marker. Two patients carried a synchronous tumor.
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We have detected microsatellite instability in bronchial mucosa at a frequency similar to that observed in tumor tissue (36% versus 33%) and associated with a normal cellular morphology. In addition, the normal samples were always taken far away from the neoplastic lesions. These observations suggest that the alterations observed were not related to the presence of neoplastic cells infiltrating the bronchial tissue but are very early changes accompanying an initial clonal expansion of cells still phenotypically normal. In particular, the analysis of microsatellite instability in tumor and bronchial DNA pairs of the same patients revealed that in one-half of the cases showing microsatellite alterations in bronchial tissue, the same shifted allelic pattern of instability was present in both bronchial and tumor DNAs. C, cases showing microsatellite alterations in bronchial mucosa but not in tumor DNAs. D, microsatellite alterations in bronchial, tumor, and sputum DNAs of cases 4 and 7.

Fig. 1. Analysis of microsatellite polymorphisms in paired normal (N), bronchial mucosa (B), and tumor (T) tissues from seven representative lung cancer patients. Arrowheads, novel alleles. A, microsatellite alterations in tumor DNAs. Partial or complete loss of one allele is also detectable in cases 1 and 2, respectively. B, patients showing the same pattern of microsatellite instability in both bronchial and tumor DNAs. C, cases showing microsatellite alterations in bronchial mucosa but not in tumor DNAs. D, microsatellite alterations in bronchial, tumor, and sputum DNAs of cases 4 and 7.

Acknowledgments

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References

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