Frequent Microsatellite Instability in Primary Small Cell Lung Cancer

Adrian Merlo, Mack Mabry, Edward Gabrielson, Robin Vollmer, Stephen B. Baylin, and David Sidransky

Division of Head and Neck Cancer Research, Departments of Otolaryngology (A. M., D. S.), Oncology (D. S., M. M., S. B. B.), and Pathology (E. G.), Johns Hopkins University, School of Medicine, Baltimore, Maryland 21205, and Department of Pathology, Duke University, Durham, North Carolina 27706 (R. V.)

Abstract

Alterations in microsatellite sequences characterize hereditary nonpolyposis colorectal cancer. This microsatellite instability is due in some kindreds to a germline mutation of the mismatch repair gene hMSH2 on chromosome 2p. Although microsatellite alterations have been reported in other hereditary nonpolyposis colorectal cancer-associated tumors including endometrial and gastric cancers, such changes were not detected in most other major neoplasms. We found that 15 of 33 (45%) primary small cell lung cancers, tumors not found in the hereditary nonpolyposis colorectal cancer syndrome, displayed alterations of microsatellite loci which consisted of deletions or expansions of (CA)n dinucleotide repeats. In 8 of these 15 neoplasms, microsatellite instability was detected in more than 10% of all tested alleles. However, small cell lung cancers that revealed instability contained widespread allelic loss and had a uniformly poor prognosis. These results expand considerably the known spectrum of tumors with microsatellite instability.

Introduction

Human cancers develop through an accumulation of somatic genetic changes during histopathological progression (1, 2). The molecular causes of these changes are poorly understood, and the contribution of exogenous versus spontaneous mutagenesis remains uncertain. A mutator phenotype leading to intrinsic genomic instability has been proposed as an early step in carcinogenesis (3, 4). The molecular causes of these changes are poorly understood, and the contribution of exogenous versus spontaneous mutagenesis remains uncertain. A mutator phenotype leading to intrinsic genomic instability has been proposed as an early step in carcinogenesis (3, 4). The molecular causes of these changes are poorly understood, and the contribution of exogenous versus spontaneous mutagenesis remains uncertain. A mutator phenotype leading to intrinsic genomic instability has been proposed as an early step in carcinogenesis (3, 4). The molecular causes of these changes are poorly understood, and the contribution of exogenous versus spontaneous mutagenesis remains uncertain. A mutator phenotype leading to intrinsic genomic instability has been proposed as an early step in carcinogenesis (3, 4). The molecular causes of these changes are poorly understood, and the contribution of exogenous versus spontaneous mutagenesis remains uncertain. A mutator phenotype leading to intrinsic genomic instability has been proposed as an early step in carcinogenesis (3, 4).

Materials and Methods

Thirty-three samples of SCLC and corresponding normal tissue were obtained from paraffin-embedded tissue derived from autopsy cases. Thirty of these patients were male and were included in a clinical study coordinated at Duke University Medical Center. In addition, one male and two female patients with SCLC were analyzed from autopsy samples from the Francis Scott Key Medical Center, Johns Hopkins School of Medicine, Baltimore. The mean survival of all patients was limited to 169.0 days (single SD, 144.4); the mean age of all patients was 58.8 years (single SD, 10.3). Mean age and survival did not differ between cases with and without microsatellite instability. Most of these patients received some form of cytotoxic and/or radiotherapeutic treatment (Table 1).

Lung cancer, the major cause of cancer-related death in Western societies (15), is comprised of a group of four histologically distinct types. Three of these, adenocarcinoma, squamous cell carcinoma, and large cell carcinoma, are considered clinically and biologically distinct from the fourth major class, SCLC. In SCLC, allelic loss occurs at high frequency at chromosomes 3p, 5q, 13q, and 17p (16–20). Major tumor types including non-SCLC were recently shown to lack genomic instability typically found in HNPCC-related tumors (21). We have found that many small cell lung cancers display widespread microsatellite alterations potentially constituting a distinct RER phenotype.

Results

To investigate genetic changes in these tumors, we scanned chromosomes 3p and 17p for allelic loss using five highly polymorphic microsatellite markers. In this approach, simple repetitive DNA sequences are amplified by PCR enabling comparison between polymorphic parental alleles in the DNA of normal and tumor samples. During this analysis, we detected distinct microsatellite alterations in tumor DNA. Tumors SC24, SC25, and SC27 revealed significant alterations of the marker CHRB/ (Fig. 1). These alterations appeared to reflect amplification or deletion of DNA within interspersed repeat elements of the form (CA)m(GT)n in repetitive DNA sequences (25, 26). Alterations in allelic size did not seem...
to be due to specific therapeutic modalities since they were also detected in patients with both limited and extensive disease who did not undergo any form of therapy (Table 1).

Differences between normal and tumor DNA banding patterns in D9S156 and IFN-a (SCJ2) revealed novel bands rather than as a "ladder" of new alleles commonly seen in independent PCR reactions and separate gel loadings. The consistent occurrence of identical alleles with concordant size between corresponding normal and tumor DNA at other microsatellite markers ruled out errors such as tissue contamination or misnumbering.

We then examined DNA from these neoplasms for genetic alterations at an additional 28 loci on chromosomes 2, 5, 6, 9, and 13. Differences between normal and tumor DNA banding patterns in microsatellite loci were detected in 15 of 33 (45%) tumors examined (Table 1). These microsatellite alterations often appear as new single 2-base pair repeat (Fig. 1). To exclude technical artifacts or specimen contamination, these alterations were reproduced in multiple PC reactions and separate gel loadings. The consistent occurrence of identical alleles with concordant size between corresponding normal and tumor DNA at other microsatellite markers ruled out errors such as tissue contamination or misnumbering.

In HNPCC-associated tumors with a RER phenotype, allelic loss occurs at a significantly lower rate as compared to non-RER tumors (6, 7). In contrast, SCLCs frequently contained instability of microsatellite repeats in chromosomal arms displaying LOH at flanking markers. Analysis of all the markers tested including loci on chromosomes 2, 3, 5, 6, 9, 13, and 17 revealed the highest percentage of LOH on 3p, 5q, 13q and 17p; further analysis revealed that LOH appeared at a similar frequency in both RER and non-RER tumors (Table 2).

### Table 1: Clinical data in primary SCLC with microsatellite instability

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Tumor grade</th>
<th>Treatment</th>
<th>Altered loci/ tested loci(%)</th>
<th>Altered loci</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC24</td>
<td>55</td>
<td>Limited</td>
<td>CAV (5X)</td>
<td>11/25 (44)</td>
<td>D152S25, D2S162, D9S (144, 162, IFN-a, 171, 200, 105), D13S170, CHRRN1, D17S786</td>
</tr>
<tr>
<td>SC27</td>
<td>55</td>
<td>Extensive</td>
<td>CAV (2X), PCI 5000 rad</td>
<td>8/20 (40)</td>
<td>D152S25, D2S162, D9S (144, 162, 171, 176), D13S170, CHRRN1, D17S786</td>
</tr>
<tr>
<td>SC25</td>
<td>63</td>
<td>Extensive</td>
<td>CAV (3X)</td>
<td>9/25 (36)</td>
<td>D152S25, D2S162, D9S (144, 162, IFN-a, 171, 200, 105), D13S170, CHRRN1, D17S786</td>
</tr>
<tr>
<td>SC12</td>
<td>68</td>
<td>Limited</td>
<td>2000 rad</td>
<td>5/24 (21)</td>
<td>D9S156, D9S162, IFN-a, D9S126, D9S166</td>
</tr>
<tr>
<td>SC18</td>
<td>90</td>
<td>Extensive</td>
<td>2400 rad</td>
<td>4/20 (20)</td>
<td>D2S162, THRB, D3S1284, D3S1007</td>
</tr>
<tr>
<td>SC17</td>
<td>57</td>
<td>Extensive</td>
<td>None</td>
<td>3/21 (14)</td>
<td>THRB, D3S421, D9S1516</td>
</tr>
<tr>
<td>SC30</td>
<td>62</td>
<td>Extensive</td>
<td>CAV (2X)</td>
<td>3/25 (12)</td>
<td>D3S1284, D9S162, D17S786</td>
</tr>
<tr>
<td>SC6</td>
<td>61</td>
<td>Extensive</td>
<td>CAV (2X)</td>
<td>2/19 (11)</td>
<td>D9S156, GSN</td>
</tr>
<tr>
<td>SC22</td>
<td>60</td>
<td>Limited</td>
<td>CAV (6X), VP-16, HMM (5X)</td>
<td>2/24 (8)</td>
<td>D9S162, D9S105</td>
</tr>
<tr>
<td>SC23</td>
<td>50</td>
<td>Extensive</td>
<td>2000 rad</td>
<td>2/24 (8)</td>
<td>D3S1284, D9S105</td>
</tr>
<tr>
<td>SC33</td>
<td>58</td>
<td>Limited</td>
<td>CAV (6X), VP-16, HMM (5X)</td>
<td>2/24 (8)</td>
<td>D3S1284, D9S1516</td>
</tr>
<tr>
<td>SC5</td>
<td>71</td>
<td>Extensive</td>
<td>CAV (2X), 2250 rad</td>
<td>1/25 (4)</td>
<td>D9S156</td>
</tr>
<tr>
<td>SC28</td>
<td>35</td>
<td>Extensive</td>
<td>None</td>
<td>1/25 (4)</td>
<td>D3S1007</td>
</tr>
<tr>
<td>SC29</td>
<td>68</td>
<td>Extensive</td>
<td>CAV (6X), 2 x 3000 rad (PCI, pelvis)</td>
<td>1/24 (4)</td>
<td>D9S126</td>
</tr>
<tr>
<td>SC31</td>
<td>63</td>
<td>Limited</td>
<td>VP-16, cisplatin (1X)</td>
<td>1/24 (4)</td>
<td>D17S786</td>
</tr>
</tbody>
</table>

* Tumors are listed in descending order of their frequency of microsatellite alterations.

Abbreviations used are: CAV = cyclophosphamide-adriamycin-vincristine, VP-16 = etoposide, HMM = hexa-methylmelamine, PCI = prophylactic cranial irradiation.

### Discussion

In this study, we found that microsatellite instability occurred commonly in SCLC. The percentage of RER tumors (45%) surpasses the incidence of similar RER-sporadic colorectal cancers (13–15%; Refs. 6, 7, and 20), thus expanding the scope of the mutator phenotype in human neoplastic diseases. Since lung cancer is not generally associated with HNPCC, this genotypic alteration may represent a mutator phenotype distinct from tumors of the Lynch syndrome, which includes cancers of the endometrium, stomach, biliopancreatic system, and urinary tract. SCLC patients are not known to be part of inherited cancer syndromes, and most are older than HNPCC patients.

SCLCs appear to give rise to a quantitatively less severe phenotype of instability (altering 4–44% of all tested alleles) as opposed to HNPCC cancers (6), which alter approximately 70% of all dinucleotide alleles at a similar frequency in both RER and non-RER tumors (Table 2). These microsatellite alterations often appear as new single bands (Fig. 1) rather than as a "ladder" of new alleles commonly seen in HNPCC tumors (6). In sporadic colorectal carcinomas with the RER phenotype, microsatellite instability is inversely correlated with allelic loss (6, 7). However, in SCLC, a high frequency of allelic loss

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**Fig. 1.** Dinucleotide repeat polymorphisms in normal (N) and tumor (T) tissue from five representative SCLC patients. Patient numbers are shown above the lanes. Normal alleles are represented by a major band surrounded by minor bands. The markers D9S162 (SC 24 and SC25), CHRRN1, (SC 24, SC25, and SC 27), D152S25 (SC25), GSN (SC6), and D9S156 and IFN-α (SC12) revealed novel bands (arrows), representing altered (deleted or expanded) alleles. LOH was found for the markers D17S786 (SC 24) and D3S1284 (SC25 and SC 27), whereas D6S105 (SC6 and SC12) showed retention of both alleles.
is detected in all tumors, including those with instability (Table 2). Furthermore, colorectal cancer patients with RER cancers have a better prognosis than non-RER tumors (5, 7, 9). SCLC patients have a very limited survival, irrespective of microsatellite instability.

Linkage analysis recently demonstrated association of HNPCC with polymorphic markers on chromosome 2p (8), and somatic as well as germline mutations of the mismatch repair gene hMSH2 at this locus were identified in RER tumors (10, 11). Microsatellite instability was also detected in sporadic colorectal (5, 7), gastric (27, 28), and endometrial cancers (29), tumor types not associated with the Lynch syndrome that probably contain mutations of the hMSH2 or a related gene. Besides the rare occurrence of microsatellite instability in bladder cancer from extended HNPCC families (30), this particular genetic alteration has not been detected in 500 sporadic human tumors including breast cancer, testicular carcinoma, and non-small cell lung cancers (21).

The high frequency of LOH at chromosome 3p (100%) in RER tumors is intriguing in that a second linkage group of HNPCC was mapped to 3p21–23 (31). However, both RER and non-RER SCLCs have a high percentage of loss at 3p, and the presence of other suppressor loci on 3p confounds interpretations of this finding (32, 33). LOH at 2p was found in 4 of 14 (29%) informative RER-SCLCs. Recent experiments have shown that inactivation of both copies of the hMSH2 gene are required in RER eukaryotic cell lines to express the mutator phenotype since one functional gene still provides sufficient enzymatic activity to prevent hypermutability of microsatellite loci (12).

Our extended analysis of over 5000 paired dinucleotide markers in approximately 400 tumors (10–15 markers per tumor) of the lung (non-SCLC), bladder, prostate, head and neck, and skin (all neoplasms unrelated to the Lynch syndrome) at this institution suggests that single somatic alterations are not uncommon. Although 1–3% of all cancers tested displayed a single alteration, the incidence of a new allele for a given dinucleotide locus was 0.5% (data not shown). A recent analysis of 196 non-HNPCC related tumors detected single alterations at a given locus in a similar percentage of dinucleotide repeats (0.5%) and an increased rate (0.5–2.6%) in tri- and tetranucleotide repeats (34). Previous studies have also suggested that larger tandem repeats may be more susceptible to alterations than dinucleotide repeats (35). Those SCLCs that showed a frequency of alterations below 5% of all dinucleotide loci tested may fall into this potential “background” rate. However, most RER SCLCs alter these loci 50–100-fold more frequently than other neoplasms, a phenomenon only seen previously in HNPCC-associated tumors.

Sequence analysis of the hMSH2 gene in SCLCs should prove informative. Alterations of the hMSH2 would point to an acquired mutator phenotype with some biological and clinical features distinct from HNPCC. Alternatively, inactivation of another mismatch repair pathway gene with a less severe phenotype may account for the microsatellite alterations observed in SCLCs. Mismatch repair complexes in bacteria contain several proteins including mutH, mutL, exonuclease, and DNA ligases (14). In yeast, inactivation of MSH3 (mutS homologue 3) leads to a less severe mutator phenotype than hMSH2 (36). Identification of the underlying genetic change(s) responsible for the mutator phenotype in SCLC may well illuminate the role of novel genes involved in human mismatch repair and tumorigenesis.

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References


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