p53 Mutations in Lung Cancer following Radiation Therapy for Hodgkin’s Disease

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Abstract

High risks of lung cancer occur after successful treatment of Hodgkin’s disease. In addition to tobacco smoking, other risk factors include radiotherapy, chemotherapy, and immunosuppression, although the relative contributions of each are unknown. We conducted p53 mutational analysis in second lung cancers following radiation therapy for Hodgkin’s disease in the Netherlands and in Ontario, Canada. Lung cancer tissues from 11 patients were analyzed by p53 immunohistochemistry and DNA sequence analysis. All were male cigarette smokers, all received radiation therapy, and six also received chemotherapy. The lung cancers occurred 9.8 years (mean) after treatment. Radiation doses to lung lobes that developed the tumors averaged 5.7 Gy (range, 3.7–11.7 Gy). Sequence analysis showed four missense and two silent p53 point mutations in five patients. There were four G:C→A:T transitions; three of four mutated deoxyguanines occurred on the coding strand, and one was a CpG site. There were two transversions: one G:C→C:G and one A:T→C:G. Despite moderate or heavy smoking histories in all patients, the mutational spectrum appears to differ from usual smoking-related lung cancers in which G:C→T:A transversions predominate. The absence of G:C→T:A mutations and the prominence of G:C→A:T transitions, which are characteristic of radiation and oxidative damage, suggest that radiotherapy might have caused some of the p53 mutations. These data illustrate the potential of mutation analysis to determine causes of human cancer. If confirmed in a larger series, these results imply that some radiation-induced cancers can be distinguished from those caused by other factors.

Introduction

Hodgkin’s disease patients have a 2–8-fold increased risk of developing lung cancer (1–4) related in part to the mutagenic effects of cancer treatment (5, 6). Associations with therapeutic external beam radiation and lung cancer risk have been reported for patients with Hodgkin’s disease (7, 8) and breast cancer (9, 10), further strengthening this association. The role of smoking in pulmonary neoplasia complicates the analysis of risk factors such as radiation. Epidemiological studies suggest that tobacco and radiotherapy may interact in a more than additive manner to produce lung cancer after Hodgkin’s disease (8) and breast cancer (9, 10), although the evidence is not strong (11, 12). Mutations driving carcinogenesis in lung cancer after Hodgkin’s disease could be generated by radiation, tobacco, oxidative damage, and/or chemotherapy in a setting of immunosuppression (2, 3, 7). The relative contributions of these factors have not been characterized.

Mutations in the p53 tumor suppressor gene occur in about one-half of human lung cancers, and most are point mutations in the highly conserved, DNA-binding domain (reviewed in Refs. 13, 14). In tobacco-related lung cancers, these mutations are predominantly G:C→T:A transversions on the nontranscribed, DNA-coding strand (13, 15). Despite extensive smoking histories, lung cancers in uranium miners exposed to radon gas have different mutation patterns than those seen in heavy smokers; these mutations may vary with the amount of radon gas exposure (16, 17). These data suggest that radiation might alter the p53 mutation spectrum, despite the influence of a potent carcinogen, such as tobacco smoke. To determine whether a distinctive mutation profile occurred among patients who develop lung cancer after external beam radiotherapy for Hodgkin’s disease, we studied all available histopathological material for patients identified in two cohort studies. The mutational spectrum observed within our series suggests that radiation may cause genetic damage that initiates lung cancer after treatment for Hodgkin’s disease.

Materials and Methods

All available tissue samples were obtained from second primary lung cancer cases from the Netherlands and Ontario. These subjects were included in prior studies of Hodgkin’s disease (4, 8, 18). For this study, the tissue samples came from male cigarette smokers, 8 of 31 cases from the Netherlands and 3 of 14 from Ontario. Only 11 (24%) of 45 tumors in the combined series could be examined because many tumors were unresectable, and many diagnostic biopsy and cytology samples are too small to be fully tested using current methods. All received radiation therapy, and six also received chemotherapy; the lung cancers occurred 9.8 years (mean) after treatment. In the
**Table 1** Lung cancer following treatment for Hodgkin’s disease: patient summaries

<table>
<thead>
<tr>
<th>Pt.</th>
<th>Age/sex</th>
<th>HD diagnosis</th>
<th>Initial therapy</th>
<th>Subsequent therapy</th>
<th>Total radiation dose (Gy) to lung</th>
<th>Interval between HD and lung cancer (yrs)</th>
<th>Site of lung cancer</th>
<th>Histology</th>
</tr>
</thead>
<tbody>
<tr>
<td>1&lt;sup&gt;+&lt;/sup&gt;</td>
<td>40/Male</td>
<td>I</td>
<td>5/73</td>
<td>RT: anterior and posterior upper mantle, abdomen</td>
<td>None</td>
<td>4.6</td>
<td>2.8</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>57/Male</td>
<td>II</td>
<td>11/66</td>
<td>RT: anterior and posterior chest, RT: left axilla</td>
<td>RT: anterior and posterior epigastrium RT: anterior and posterior pelvis VLB, 6 months CTX, VLB and PROC + 17 cycles CTX, 44 months</td>
<td>5.6</td>
<td>10</td>
<td>Right upper lobe</td>
</tr>
<tr>
<td>3</td>
<td>33/Male</td>
<td>III</td>
<td>7/74</td>
<td>RT: Anterior and posterior mantle and abdomen MOPP, 6 cycles</td>
<td>None</td>
<td>5.8</td>
<td>7.3</td>
<td>Left upper lobe</td>
</tr>
<tr>
<td>4</td>
<td>26/Male</td>
<td>III</td>
<td>4/67</td>
<td>RT: mantle, inverted Y, inguinal (left and right)</td>
<td>MOPP, 8 cycles</td>
<td>5.1</td>
<td>12.2</td>
<td>Right upper lobe</td>
</tr>
<tr>
<td>5</td>
<td>49/Male</td>
<td>II</td>
<td>1/72</td>
<td>RT: mantle, inverted Y</td>
<td>None</td>
<td>4.5</td>
<td>8.1</td>
<td>Right upper lobe</td>
</tr>
<tr>
<td>6</td>
<td>56/Male</td>
<td>I</td>
<td>2/73</td>
<td>RT: mantle, para-aortic, spleen + splenic hilum</td>
<td>None</td>
<td>5.6</td>
<td>9.5</td>
<td>Right upper lobe</td>
</tr>
<tr>
<td>7</td>
<td>38/Male</td>
<td>N/A&lt;sup&gt;+&lt;/sup&gt;</td>
<td>6/62</td>
<td>RT: cervical (left); axillary (left)</td>
<td>Cervical (right), mantle, para-aortic, spleen and splenic hilum MOPP, 7 cycles</td>
<td>6.0</td>
<td>17.8</td>
<td>Right bronchus</td>
</tr>
<tr>
<td>8</td>
<td>47/Male</td>
<td>III</td>
<td>12/80</td>
<td>RT: Waldeyer ring, cervical (left + right), jugular + mediastinal, para-aortic + spleen + splenic hilum MOPP, 3 cycles CTX, ADR, TENI + 3 cycles</td>
<td>None</td>
<td>3.7</td>
<td>9.3</td>
<td>Right upper lobe</td>
</tr>
<tr>
<td>9</td>
<td>24/Male</td>
<td>I</td>
<td>11/65</td>
<td>RT: cervical (left)</td>
<td>RT: cervical (left and right), axillary (left), mediastinal</td>
<td>4.3</td>
<td>15.3</td>
<td>Right upper lobe</td>
</tr>
<tr>
<td>10</td>
<td>50/Male</td>
<td>I</td>
<td>9/80</td>
<td>RT: mantle, para-aortic, spleen + splenic hilum</td>
<td>None</td>
<td>11.7</td>
<td>1.3</td>
<td>Left upper lobe</td>
</tr>
<tr>
<td>11</td>
<td>46/Male</td>
<td>II</td>
<td>10/74</td>
<td>RT: mantle, para-aortic, spleen + splenic hilum</td>
<td>RT: L4-L5, iliac (left) VLB + PROC</td>
<td>6.6</td>
<td>14.3</td>
<td>Left upper lobe</td>
</tr>
</tbody>
</table>

<sup>a</sup> HD, Hodgkin’s disease; CTX, cyclophosphamide; MOPP, mechloethamine, oncovin, procarbazine, prednisone; RT, radiotherapy; SCCL, small cell lung cancer; SCCA, squamous cell carcinoma; TENI, teniposide; VLB, vinblastine.

<sup>b</sup> Age at diagnosis of Hodgkin’s disease.

<sup>c</sup> Dose to lobe of lung in which second primary cancer later occurred. Radiotherapy was administered by Cobalt-60 (Patients 1–4), megavoltage (patients 5, 6, 8–11), or orthovoltage and megavoltage (Patient 7).

<sup>d</sup> Patients 1–3 were contributed by the Ontario Cancer Treatment and Research Foundation, Toronto, Ontario; Patients 4–11 were contributed by the Netherlands Cancer Institute, Amsterdam, The Netherlands.

<sup>e</sup> —, Site unknown. Each lobe of lung had received 4–6 Gy.

<sup>f</sup> N/A, not available.

Netherlands series (2), 31 lung cancers developed among 1939 patients versus 8.3 expected, indicating a nearly 4-fold excess over the general population expectation.

Radiation dose to lobe of lung was estimated for each patient in whom a second cancer developed, using details of radiotherapy abstracted from the treatment record. In regions outside the radiation beam, absorbed dose to the lung was calculated, based on measurements in water phantoms (19). For regions inside a treatment beam, doses were calculated using conventional treatment planning techniques. All dose estimates took account of lung blocking where appropriate. Data on treatment fields, radiation energies, absorbed dose to lung, interval between Hodgkin’s disease and lung cancer, and other pertinent clinical information are summarized in Table 1.

**Immunohistochemistry.** Immunohistochemical analysis was performed using conventional peroxidase methods as reported previously (20). Tissue sections were incubated overnight at 4°C with a saturating dilution of a polyclonal rabbit antiserum, CM-1, raised against full-length p53 protein (21). Localization of the primary antibody was detected by an avidin-biotin peroxidase system (Vectastain Elite kit; Vector Laboratories, Burlingame, CA). A positive control section was included in each experiment; nonneoplastic tissues within each section served as internal negative controls. Results were analyzed semiquantitatively and divided into four groups: Neg, no nuclear staining present; 1+, <10% of tumor nuclei stained; 2+, 10–70% of tumor cell nuclei stained; and 3+, >70% of tumor nuclei stained.

**Genomic DNA Extraction, p53 Amplification, and Sequence Analysis.** Paraffin-embedded tumor samples were de-waxed and microdissected from 50-μm sections. Genomic DNA was isolated by SDS/proteinase K treatment (final concentration, 1% SDS and 0.5 mg/ml of proteinase K) at 50°C for 12 h, followed by phenol-chloroform extraction, ethanol precipitation, and resuspension in 50 μl of sterile water. Nonneoplastic tissues flanking the tumor were isolated separately for germline analysis.

The most commonly mutated coding sequences (i.e., exons 5–8) were analyzed in all 11 tumors, and the occasionally altered exons (i.e., exons 4, 9, and 10) were examined in tumor samples that were positive by immunohistochemistry but negative for mutation in exons 5–8. For each sample, there were two
The mutation at codon I75 was homozygous, indicating that the second allele was deleted. All others appeared heterozygous, although contamination with normal stroma could not be excluded as a source of the wild-type sequence. Quantitative smoking history was not available for patient 3, who was described as a "heavy, long-term smoker." In patients 1, 8, 9, and 10, sequence analysis included exons 4, 9, and 10. Smoking histories for two patients were provided only as cigarettes/day: duration of smoking was not provided.

Heterozygous polymorphisms at codons 72 (CGC→5, exon 2) and 293 (GGG→GGA, exon 6) were detected in patients 10 and 5, respectively. The mutation at codon 175 was homozygous, indicating that the second allele was deleted. All others appeared heterozygous, although contamination with normal stroma cannot be excluded as a source of the wild-type sequence.

Results

Lung cancers from five Hodgkin’s disease survivors contained six somatic, point mutations (four missense and two silent mutations); there were four G:C→A:T transitions plus one G:C→C:G and one A:T→C:G transversions (Table 2). Germ-line analysis of nontumor samples showed that all mutations were somatic alterations. Missense and silent mutations occurred at codons 278 and 293, respectively, in the tumor from patient 5. Clonal analysis indicated that both base substitutions occurred on the same allele.

Immunohistochemical analysis showed nuclear accumulation of p53 protein in tumor tissues from 7 patients (Table 2). Tumors from patients 2, 5, and 6 were positive by immunohistochemistry and contained missense mutations, but four tumors which were positive by immunohistochemistry contained wild-type sequence in the commonly mutated regions within exons 5–8. Because 15% of lung cancer mutations occur outside exons 5–8 (14), exons 4, 9, and 10 were examined in the four patients (1, 8, 9, and 10) who were positive for immunohistochemistry and negative for mutation. A missense mutation in exon 4 (codon 89) was identified in patient 9. The mechanisms for protein accumulation in patients 1, 8, and 10 are probably nonmutational; possibilities include binding and stabilization by DNA tumor virus proteins or cellular proteins such as mdm-2 (25).

Discussion

We report the first p53 mutational spectrum analysis in lung cancers after radiotherapy for Hodgkin’s disease. The principal finding is an unusual mutational profile dominated by G:C→A:T transitions at non-CpG sites, which are characteristic of radiation or oxidative damage (reviewed in Refs. 26, 27). Despite moderate or heavy smoking histories in all 11 subjects, these data suggest that radiation was the primary mutagen and that smoking, chemotherapy, and other influences were cofactors in the development of these secondary lung cancers. Although all lung cancers occurred an average of 9.8 years after treatment, for patients 1 and 10, latencies were unusually short, i.e., 2.8 and 1.3 years, respectively. However, neither of these patients had a p53 mutation, so that excluding these two cases would not change our results, except to increase the mutation frequency within the residual cohort. Because of the short latency interval and the absence of G:C→A:T transitions in patients 1 and 10, we do not believe that these two lung cancers were radiogenic, and we point out that both patients were heavy smokers.

In tobacco-related lung tumors, the most common p53 base changes are G:C→T:A transversions, with a strand bias in which the mutated guanine typically occurs on the nontranscribed, DNA-coding strand (see Table 3; Ref. 13). The frequency of G:C→T:A transversions increases with increasing smoking history, and conversely, the frequency of G:C→A:T transitions significantly decreases (15). Although all 11 patients in this series were moderate or heavy smokers, not one G:C→T:A base change was detected, and 4 of 6 mutations were G:C→A:T transitions (patients 5, 6, 9, and 11). Three of five mutated guanine residues occurred on the coding strand, and only one of four G:C→A:T transitions occurred at a CpG site. This mutation profile appears to differ from smoking-related lung cancers in which G:C→A:T base changes at non-CpG sites are much more common. 2

Table 2 p53 analysis in lung cancers after radiation therapy for Hodgkin’s disease

<table>
<thead>
<tr>
<th>Patient</th>
<th>Smoking history (pack-yrs)</th>
<th>Histology</th>
<th>p53 IHC*</th>
<th>p53 sequence analysis</th>
<th>Codon</th>
<th>Base change</th>
<th>CpG site</th>
<th>Amino acid change</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&gt;2 pack-yrs</td>
<td>SCCA</td>
<td>3+</td>
<td>WT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1-2 pack-yrs</td>
<td>SCCA</td>
<td>2+</td>
<td>CTG→CGG</td>
<td>145</td>
<td>No</td>
<td>Leu→Arg</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Heavy, long-term†</td>
<td>SCCA</td>
<td>Neg</td>
<td>WT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>31</td>
<td>SCCA</td>
<td>Neg</td>
<td>WT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5†</td>
<td>48</td>
<td>SCCA</td>
<td>3+</td>
<td>CCT→CTG</td>
<td>278</td>
<td>No</td>
<td>Pro→Arg</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>18</td>
<td>SCCA</td>
<td>2+</td>
<td>GGG→GGA</td>
<td>293</td>
<td>No</td>
<td>Gly→Gly</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>27</td>
<td>SCLC</td>
<td>Neg</td>
<td>CGC→CAC</td>
<td>175†</td>
<td>Yes</td>
<td>Arg→His</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>35</td>
<td>Adenoca</td>
<td>3+</td>
<td>WT†</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>25</td>
<td>SCCA</td>
<td>3+</td>
<td>CCC→TCC</td>
<td>89†</td>
<td>No</td>
<td>Pro→Ser</td>
<td></td>
</tr>
<tr>
<td>10†</td>
<td>19</td>
<td>Adenoca</td>
<td>2+</td>
<td>WT†</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>35</td>
<td>SCCA</td>
<td>Neg</td>
<td>GAG→GAA</td>
<td>285</td>
<td>No</td>
<td>Glu→Glu</td>
<td></td>
</tr>
</tbody>
</table>

* IHC: immunohistochemistry; SCCA, squamous cell carcinoma; SCLC, small cell lung carcinoma; adenoca, adenocarcinoma; pack, packs per day; WT, wild type; Neg, negative.
† Smoking histories for two patients were provided only as cigarettes/day: duration of smoking was not provided.
‡ In patients 1, 8, 9, and 10, sequence analysis included exons 4, 9, and 10.
§ Quantitative smoking history was not available for patient 3, who was described as a "heavy, long-term smoker."
¶ Heterozygous polymorphisms at codons 72 (CGC→5, exon 2) and 293 (GGG→GGA, exon 6) were detected in patients 10 and 5, respectively.
‡ The mutation at codon 175 was homozygous, indicating that the second allele was deleted. All others appeared heterozygous, although contamination with normal stroma cannot be excluded as a source of the wild-type sequence.

2 CpG site is a shorthand notation for a cytosine linked to a guanine by a phosphodiester bond. The significance in this context is that cytosine bases within CpG sites are methylated frequently at the 5′ position. 5-Methyl-cytosine is susceptible to spontaneous deamination, which produces a base change of cytosine to thymidine. This is a form of spontaneous or endogenous mutation that does not require an exogenous mutagen.
Table 3  p53 Mutational spectra in lung cancers from Hodgkin's disease patients, uranium miners, tobacco smokers, and in vitro model systems

<table>
<thead>
<tr>
<th>Human lung cancers</th>
<th>Genetic target sequence</th>
<th>Primary radiation class</th>
<th>n</th>
<th>G:C</th>
<th>A:T</th>
<th>C:G</th>
<th>Deletion/insertion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post-Hodgkin's</td>
<td>p53</td>
<td>y</td>
<td>6</td>
<td>67%</td>
<td>0</td>
<td>17</td>
<td>0</td>
</tr>
<tr>
<td>Uranium miners</td>
<td>p53</td>
<td>y</td>
<td>6</td>
<td>12.5</td>
<td>37.5</td>
<td>12.5</td>
<td>0</td>
</tr>
<tr>
<td>Uranium miners+</td>
<td>p33 α</td>
<td>30</td>
<td>17</td>
<td>6.7</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Smokers</td>
<td>p53</td>
<td>None</td>
<td>502</td>
<td>24</td>
<td>40</td>
<td>9</td>
<td>3</td>
</tr>
</tbody>
</table>

*Percentage of mutations that are G:C→A:T transitions at CpG dinucleotides.*

1. Three CpG guanines occurred on the coding strand; one of four was a CpG site.
2. *P = 0.10, FET (Fisher's Exact Test); comparison of G:C→A:T frequency among post-Hodgkin's lung cancers and smokers with lung cancer.*
3. Uranium miners from New Mexico, reported by Vahakangas et al. (116).
4. Uranium miners from the Colorado plateau, reported by Taylor et al. (17).
5. 16 of 19 G:C→T:A transversions occurred at codon 249, AGG→ATG (14).
6. Composite data from Greenblatt et al. (14).
7. Composite data from Refs. 41-44; radiation doses were 2 Gy. All studies used human B-lymphoblastoid cell lines.
8. *HPRT, hypoxanthine-guanine phosphoribosyl transferase gene, NR, not reported.*
9. Composite data from Refs. 28, 29, 45-47; all studies used human lymphoblast cells and shuttle plasmid, pZ189, containing *E. coli supF* gene as mutational target. Radiation doses were 5-160 Gy.

CpG sites account for only 15% of p53 mutations (*P = 0.10, Fisher's Exact Test; Table 3; reviewed in Ref. 14).

Related data come from analyses of lung cancers from uranium miners exposed to radon, an α emitter. In these tumors, the p53 mutational spectrum is also remarkably different from that observed in tobacco-related lung tumors, despite heavy smoking histories among these subjects (16, 17). Among uranium miners from New Mexico, 3 of 6 base substitutions were G:C→T:A, but no guanine occurred on the nontranscribed, DNA-coding strand (16). The most striking observation is a mutational hotspot at the second position of codon 249, AGG→ATG (17) which occurred among Colorado plateau miners with very high level exposure to radon (*i.e.*, >1000 Working Level Month). Although this site is a minor mutational hotspot in smoking-related lung cancers (14), 16 of 25 (64%) base changes reported in lung tumors from Colorado plateau miners were G:C→T:A transversions at this codon (17). Mutation hotspots are characteristic of radiation mutagenesis (28, 29); typical mechanisms include sequence context and gene structure/function features. It is possible that the differences in mutation spectrum between the uranium miners and Hodgkin's disease patients can be explained by factors including variation in amount of absorbed radiation, different types of radiation (*γ* versus α), temporal pattern, and modality of exposure (several high-dose fractions over 6 weeks versus low-dose, chronic exposure over many years), genetic background, and environmental factors.

Although the mutational spectra of ionizing radiation vary depending on target locus, chromosomal context to locus, dose, dose rate, and DNA repair mechanism, large and small deletions are the most common lesions (reviewed in Refs. 30, 31). However, several *in vitro* model systems have reported base substitutions and many show a predominance of G:C→A:T transitions. We reviewed data generated by two model systems, the *HPRT* locus and a shuttle vector (Table 3); both systems use human cells to avoid interspecies differences in DNA repair. Mutations in the *HPRT* locus are selected by their interruption of the coding sequence (further described in Ref. 31); this explains the predominance of deletions and insertions, which account for 83% of lesions (see Table 3). By using γ-ray doses of 2 Gy, the *HPRT* base substitutions include all mutation classes with a slight excess of G:C→A:T transitions. The second system uses the pZ189 shuttle vector with the *E. coli supF* gene as a mutational target. The architecture of this plasmid is biased toward base substitutions (89%) and against insertions and deletions (11%); described in Ref. 29); this resembles the p53 mutation profile that selects for base substitutions (reviewed in Ref. 14). γ-ray (5-160 Gy) of the shuttle vector, followed by passage through human lymphoblastoid cells for DNA repair, produced mostly G:C→A:T transitions (53%; Table 3), which are commonly attributed to the mutagenic activity of free radicals produced by the radiolysis of water (reviewed in Refs. 26, 27).

The minimum latency period for solid cancers caused by radiation alone is typically 10 years or more (32). However, the risk of lung cancer begins to increase in the 5–9-year period after diagnosis of Hodgkin's disease (4, 33), and this accelerated onset suggests an additional factor(s) expediting the process. Factors that might shorten the latency period include tobacco, immunosuppression (34), chemotherapy, and host variables. Our data are consistent with primary mutagenesis by radiation and free radicals, and statistical analysis of the patient series showed that lung cancer risk among smokers strongly correlated with increasing radiation dose (*P trend 0.01; Ref. 8,*). However, it is also possible that radiation and tobacco may interact to cause the unusual p53 mutational spectrum observed in our series. Weak but supporting evidence for an interaction between these two mutagens comes from epidemiological studies of breast cancer patients (11, 35) and Hodgkin's disease patients (8) treated with radiation. Among underground miners, the interaction between radon and tobacco use is less than multiplicative but more than additive (36).

Combination chemotherapy includes many alkylating agents, including procarbazine, which is frequently used to treat Hodgkin's disease. Procarbazine commonly produces G:C→A:T transitions (37), which occurred in four tumors in our series. Among these four patients, three received radiation therapy alone, and one had radiation therapy plus vinblastine...
and procarbazine. Therefore, one of four G:C→A:T transitions might be related to procarbazine and/or radiotherapy.

Silent base changes at codons 293 and 285 of the p53 gene occurred in lung tumors from patients 5 and 11, respectively. Silent mutations do not produce an amino acid change due to degeneracy of the genetic code.

References

p53 mutations in lung cancer following radiation therapy for Hodgkin's disease.
