Frequent p53 Mutations in Head and Neck Cancer

Kenneth D. Somers, M. Anne Merrick, Martha E. Lopez, Leonard S. Incognito, Gary L. Schechter, and Graham Casey

Head and Neck Tumor Biology Program, Departments of Microbiology/Immunology [K. D. S., M. A. M., L. S. I.] and Otolaryngology—Head and Neck Surgery [K. D. S., M. A. M., G. L. S.], Eastern Virginia Medical School, Norfolk, Virginia 23501, and Department of Pediatrics, School of Medicine, University of California at Irvine, Irvine, California 92717 [M. E. L., G. C.]

ABSTRACT

Squamous cell carcinomas of the head and neck (SCCHN) are associated strongly with the use of tobacco and alcohol, but little is known about the molecular pathogenesis of these tumors. In the present study, we analyzed SCCHN for mutations in the tumor suppressor gene p53 by immunocytochemistry and complementary DNA sequencing. Overexpression of p53 protein was detected in 13 (100%) of 13 SCCHN cell lines and in tumor cells cultured directly from 10 (77%) of 13 patients with SCCHN. Direct evidence for p53 mutations was obtained by sequencing p53 complementary DNA from eight SCCHN cell lines and two tumor xenografts. The genetic alterations included seven missense mutations resulting in single amino acid substitutions, a mutation encoding a stop codon, one 10-base pair deletion, and one 2-base pair addition. All seven missense mutations were G to T transversions, five of which were clustered at codons 245 and 248. A similar high frequency of G to T transversions predominates in lung cancer, another tobacco-related disease. Mutation of the p53 gene is the most common genetic alteration detected in SCCHN and implicates this gene locus as a critical site of specific damage by mutagenic carcinogens in tobacco, one of the important risk factors in the etiology of this disease.

INTRODUCTION

SCCHN is the sixth most frequent cancer in the world; an estimated 378,500 new cases were diagnosed in 1980, the latest date for which worldwide incidence data are available (1). In the United States, an estimated 44,000 new cases were detected and nearly 12,000 deaths were reported last year (2). The overall 5-year survival rate for patients with head and neck cancer is among the lowest of the major cancers and has not changed during the past two decades. Progress in understanding the etiology and pathogenesis of head and neck tumors has been slowed by a lack of molecular studies investigating genetic alterations that underlie the development and progression of these cancers. Two of the strongest risk factors for SCCHN are use of tobacco and consumption of alcohol. Tobacco smoking is also a major risk factor in human lung and esophageal cancer. Recent evidence has demonstrated that mutations in the tumor suppressor gene p53 are common in these cancers (3–6). Tumor suppressor genes function as negative regulators of cellular proliferation. Their inactivation, either by deletion or mutation, results in loss of function and unrestrained cellular growth (7). The p53 gene is frequently lost or mutated in several kinds of tumors, including colon, lung, breast, brain, ovary, and esophagus (reviewed in Ref. 8), implicating p53 as an important tumor suppressor gene. Given the common epidemiological risk factors associated with cancers of the aerodigestive tract, we suspected that p53 mutations might be an important genetic alteration in head and neck cancers. In the present study, we used both immunocytochemistry and cDNA sequencing to assess p53 gene mutations in cell lines derived from SCCHN, cells cultured as primary explants from tumor tissue, and SCCHN xenografts in nude mice. The results demonstrate a high frequency of p53 mutations in SCCHN with preferential G to T transversions clustered at codons 245 and 248.

MATERIALS AND METHODS

Cell Lines and Tumor Samples. Squamous carcinoma cell lines derived from the larynx (UMSCC10A, UMSCC10B, UMSCC16), tongue (UMSCC19), epiglottis (UMSCC23), and facial skin (UMSCC63) were provided by T. E. Carey. A SCC of the hypopharynx (FaDu), three tongue carcinoma lines (SCC4, SCC15, SCC225), a SCC of the vulva (A431), and a SCC of the cervix (SiHa) were obtained from the American Type Culture Collection. SCC lines derived from an alveolar ridge (EVSCC1), a tonsil (EVSCC3), and a floor of the mouth (EVSCC4) tumor were established in culture by us from nude mouse xenografts. Cells were grown in Eagle’s minimal essential medium supplemented with 10% fetal bovine serum or under conditions recommended by the suppliers. Tissue specimens from patients undergoing surgery for head and neck cancer and from head and neck tumors propagated as xenografts in nude mice (9) were used to initiate primary cell cultures. Primary tumor, adjacent normal tissue, and metastases (when present) were dissociated by collagenase treatment as described previously (10), seeded into flasks precoated with type I collagen (Collagen Corp., Palo Alto, CA), and grown in serum-free PC-1 medium (Endotronics, Minneapolis, MN). Tumors were staged by the tumor-node-metastasis (TNM) staging system for classification of head and neck cancers: extent of primary tumor (T), status of regional lymph nodes (N), and absence or presence of metastatic spread (M). Regional lymph node classification was based on size (N1, N2, N3), number (single [a] or multiple [b]), and location (homolateral [N1, N2] or bilateral [N3]). TNM categories are grouped into Stages I to IV that determine treatment and prognosis.

Immunocytochemical Analysis. Cells from fresh tissue specimens or tissue culture cells were plated onto glass coverslips, fixed in 95% ethanol at room temperature for 10 min, washed with phosphate-buffered saline, and incubated at room temperature for 30 min with monoclonal antibody PAb 1801 or PAb 240 (Oncogene Science, Uniondale, NY). PAb 1801 recognizes an epitope on the NH2-terminal domain of normal mouse IgG was used as a negative control antibody. Primary antibody binding was detected by using the avidin-biotin-complex immunoperoxidase system (Vector Laboratories, Burlingame, CA), following manufacturer’s recommendations.

RNA Extraction. Total cellular RNA was prepared from tissue culture cells or tumor tissue by the guanidinium isothiocyanate/phenol/chloroform extraction method (11).

PCR Amplification and Sequencing of p53. One μg of total RNA was reverse transcribed using oligodeoxythymidylylade and Moloney-murine leukemia virus reverse transcriptase (BRL, Bethesda, MD). Following inactivation, one-tenth (2 μl) of the reaction was amplified by PCR with Taq polymerase (Perkin-Elmer Cetus, Norwalk, CT) and primers P53CL5 (5'-CACGACGGTGACACGCTTCCCTG-3') and P53CL6 (5'-CTGTGGTCCTCTCTACGACACCC-3') which encompass the entire coding domain of the p53 gene, using an Ericomp temperature cycler (Ericomp, San Diego, CA). The 1200-base pair product was...
purified by ammonium acetate precipitation, and reamplified by asymmetric PCR (12, 13), using primers P53S1 (5'-CAGACTGCCTTC-CGGGTTCAC-3') and P53AS1 (5'-GGAGGCTGTCAGTTGGGGAAC-3') at a 0.01:1 ratio. The method of asymmetric PCR and direct sequencing without a cloning step was specifically chosen to reduce the possibility of identifying false positives introduced by the PCR amplification method itself. The predominantly single-strand product was sequenced by using Centricon 100 columns (Amicon, Beverly, MA), and sequenced by the dyeideoxy chain termination method with the use of a Sequenase 2.0 kit (US Biochemicals, Cleveland, OH). Samples were reamplified and resequenced for confirmation of mutations. The following internal sequence primers were used:

For positive strand sequence:
P53S1 5'-CAGACTGCCTTC-CGGGTTCAC-3';
P53SQ5 5'-CTCCCCTGCCCTCAACAAG-3';
P53SQ1 5'-CCTGTCATCTTCTGTCCCTFCCCAG-3';
P53SQ2 5'-GGCGCATCTACAAGCAGTC-3';
P53SQ3 5'-GTGGGCTCTGACTGTACCAC-3';
P53SQ4 5'-CGAGGGAAGAGAATCTCCGC-3';
P53SQ6 5'-CTGGAGTCTTCCAGTGTG-3';
P53SQ7 5'-CTGCCTTGTGCTCCCTCGG-3';
P53SQ8 5'-CTGGAGTCTTCCAGTGTG-3';
P53SQ9 5'-CTGCCTTGTGCTCCCTCGG-3';
P53SQ10 5'-CTGGAGTCTTCCAGTGTG-3'

For negative strand sequence:
P53SQ11 5'-CTCGGACCTTCAGACTGCCT-3';
P53SQ12 5'-CTTGTCATCTTCTGTCCCTFCCCAG-3';
P53SQ13 5'-GTGGGCTCTGACTGTACCAC-3';
P53SQ14 5'-CGAGGGAAGAGAATCTCCGC-3';
P53SQ15 5'-CTGGAGTCTTCCAGTGTG-3';
P53SQ16 5'-CTGGAGTCTTCCAGTGTG-3';
P53SQ17 5'-CTGGAGTCTTCCAGTGTG-3'

The sequencing reaction mixes were separated by electrophoresis on 6% polyacrylamide gels.

RESULTS AND DISCUSSION

Clinicopathological features of the patients with SCCHN are shown in Tables 1 and 2. Patient age at tumor diagnosis ranged from 19 to 85 years. Nearly all patients had a history of chronic smoking and alcohol use. Three exceptions were patient EVSCC1, 55 years old, patient EVSCC7PX, 19 years old, and patient EVSCC17PX, 34 years old; all had no previous history of tobacco or alcohol use.

We evaluated p53 protein expression in 13 cell lines derived from SCCHN by immunocytochemical staining with anti-p53 monoclonal antibodies PAB 1801 and PAB 240. Point mutations in the p53 gene result in an increased stability of the mutant protein which can be detected by immunocytochemistry (14). This approach has been used successfully to screen for p53 mutations in breast, colorectal, and lung cancer (4, 15, 16).

Table 1 shows that all 13 of the SCCHN cell lines were positive for increased p53 expression using the two antibodies. In 11 of the cell lines, p53 immunostaining with PAB 1801 and PAB 240 was concordant, with positive cells demonstrating strong nuclear staining (Fig. 1, a and d). Two of the cell lines (SCC25, EVSCC1), did not react with PAB 1801, but stained positive with PAB 240, which recognizes a different epitope on mutant p53. Staining with PAB 240 was less intense and, in the case of EVSCC1, localized to the cytoplasm (Fig. 1e). p53 antibodies were nonreactive with SiHa cells (Fig. 1b), shown previously to contain wild-type p53 (17).

To verify that overexpression of p53 protein in SCCHN cell lines was due to a mutated p53 gene, we reverse transcribed RNA from 8 cell lines and 2 tumor xenografts, and amplified the cDNA by PCR. As a control we sequenced the cDNA from A431, an epidermoid carcinoma cell line, shown previously to contain a mutated p53 gene, CGT to CAT (Arg to His) at codon 273 (18). p53 gene mutations were detected in all 8 cell lines and both tumor xenografts (Fig. 2A). In each case analyzed, there was a direct correlation between abnormal p53 protein expression and gene mutation (Table 1). We confirmed a point mutation at codon 273 in A431 (data not shown). Six of the SCCHN cell lines and one xenograft harbored missense point mutations, all of which were G to T transversions. Five of the missense mutations resulting in amino acid substitutions were at codons 245 and 248, and one each at codons 242 and 176. UMSCC10A and UMSCC10B are a primary and recurrent laryngeal tumor, respectively, derived from the same patient. Both lines have the same p53 mutation at codon 245, indicating that the recurrent tumor was derived from the same clone. EVSCC1 had a nonsense mutation at codon 213 resulting in chain termination, and did not contain a wild-type allele (Fig. 2B). By immunostaining, p53 was specifically localized to the cytoplasm of EVSCC1 cells (Fig. 1e). This result is consistent with the deletion of a region of the p53 gene.

Table 1 Clinicalopathological features and alterations of p53 in SCCHN cell lines and tumor xenografts

<table>
<thead>
<tr>
<th>Source</th>
<th>Site</th>
<th>Age</th>
<th>Sex</th>
<th>Stage</th>
<th>TNM</th>
<th>Tobacco pack (yr)</th>
<th>p53 immunostaining</th>
<th>Codon</th>
<th>Mutation</th>
<th>Amino acid</th>
<th>Alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCCN cell lines</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FaDu</td>
<td>HPX</td>
<td>56</td>
<td>M</td>
<td>NS</td>
<td>N1</td>
<td>+</td>
<td>+</td>
<td>248</td>
<td>CCG → CTG</td>
<td>Arg → Leu</td>
<td>Hemi/homozygous</td>
</tr>
<tr>
<td>UMSCC10A</td>
<td>LX</td>
<td>57</td>
<td>M</td>
<td>III</td>
<td>T2N0M0</td>
<td>20</td>
<td>+</td>
<td>+</td>
<td>245</td>
<td>GCC → TGC</td>
<td>Gly → Cys</td>
</tr>
<tr>
<td>UMSCC10B</td>
<td>LX</td>
<td>57</td>
<td>M</td>
<td>III</td>
<td>T2N0M0</td>
<td>20</td>
<td>+</td>
<td>+</td>
<td>245</td>
<td>GCC → TGC</td>
<td>Gly → Cys</td>
</tr>
<tr>
<td>UMSCC16</td>
<td>LX</td>
<td>61</td>
<td>F</td>
<td>III</td>
<td>T2N0M0</td>
<td>60</td>
<td>+</td>
<td>+</td>
<td>248</td>
<td>CCG → CTG</td>
<td>Arg → Leu</td>
</tr>
<tr>
<td>UMSCC19</td>
<td>TG</td>
<td>66</td>
<td>M</td>
<td>III</td>
<td>T1N1M0</td>
<td>60</td>
<td>+</td>
<td>+</td>
<td>148-151</td>
<td>10-base pair deletion</td>
<td>Frame shift</td>
</tr>
<tr>
<td>UMSCC23</td>
<td>LX</td>
<td>36</td>
<td>F</td>
<td>III</td>
<td>T2N0M0</td>
<td>50</td>
<td>+</td>
<td>+</td>
<td>176</td>
<td>TGG → TTC</td>
<td>Cys → Phe</td>
</tr>
<tr>
<td>UMSCC63</td>
<td>Skin</td>
<td>82</td>
<td>M</td>
<td>NS</td>
<td>Pipe</td>
<td>+</td>
<td>+</td>
<td>242</td>
<td>TGG → TTC</td>
<td>Cys → Phe</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>SCC4</td>
<td>TG</td>
<td>55</td>
<td>M</td>
<td>III</td>
<td>T2N0M0</td>
<td>100</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCC15</td>
<td>TG</td>
<td>55</td>
<td>M</td>
<td>IV</td>
<td>T1N1M0</td>
<td>120</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCC25</td>
<td>TG</td>
<td>70</td>
<td>M</td>
<td>III</td>
<td>T1N1M0</td>
<td>75</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EVSCC1</td>
<td>AR</td>
<td>55</td>
<td>M</td>
<td>II</td>
<td>T2N0M0</td>
<td>None</td>
<td>-</td>
<td>+</td>
<td>213</td>
<td>CGA → TGA</td>
<td>Arg → stop</td>
</tr>
<tr>
<td>EVSCC3</td>
<td>TL</td>
<td>65</td>
<td>M</td>
<td>IV</td>
<td>T2N0M0</td>
<td>50</td>
<td>+</td>
<td>ND</td>
<td>273</td>
<td>CGT → CAT</td>
<td>Arg → His</td>
</tr>
<tr>
<td>EVSCC4</td>
<td>FOM</td>
<td>45</td>
<td>M</td>
<td>III</td>
<td>T1N1M0</td>
<td>30</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control cell lines</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A431</td>
<td>VUL</td>
<td>85</td>
<td>F</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td>273</td>
<td>CGT → CAT</td>
<td>Arg → His</td>
</tr>
<tr>
<td>SiHa</td>
<td>CX</td>
<td>55</td>
<td>F</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td>273</td>
<td>CGT → CAT</td>
<td>Arg → His</td>
</tr>
<tr>
<td>Tumor xenografts</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EVSCC3X</td>
<td>TL</td>
<td>65</td>
<td>M</td>
<td>IV</td>
<td>T2N0M0</td>
<td>50</td>
<td>ND</td>
<td>248</td>
<td>CCG → CTG</td>
<td>Arg → Leu</td>
<td>Hemi/homozygous</td>
</tr>
<tr>
<td>EVSCC4X</td>
<td>FOM</td>
<td>45</td>
<td>M</td>
<td>IV</td>
<td>T1N1M0</td>
<td>20</td>
<td>289-290</td>
<td>2-base pair (TT)</td>
<td>Frame shift</td>
<td>Hemi/homozygous</td>
<td></td>
</tr>
</tbody>
</table>

* Tumor sites: hypopharynx (HPX), larynx (LX), tongue (TG), alveolar ridge (AR), tonsil (TL), floor of mouth (FOM), vulva (VUL), cervix (CX).

** Packs/day × years smoked.

* Staining is defined as (+) strongly positive nuclear staining in all cells; (±) nuclear staining in some cells, (−) no staining.

*NS* = not staged; *NI* = no information; *ND* = not determined.

* Cytoplasmic staining.
Table 2 Clinicopathological features and p53 overexpression in primary explant cultures of SCCHN

<table>
<thead>
<tr>
<th>Source</th>
<th>Sitea</th>
<th>Age</th>
<th>Sex</th>
<th>Stage</th>
<th>TNM</th>
<th>Tobacco pack (yr)</th>
<th>p53 immunostaining</th>
</tr>
</thead>
<tbody>
<tr>
<td>EVSCC5PX</td>
<td>TL</td>
<td>63</td>
<td>M</td>
<td>III</td>
<td>T1N0M0</td>
<td>80</td>
<td>+</td>
</tr>
<tr>
<td>EVSCC6PX</td>
<td>TG</td>
<td>85</td>
<td>F</td>
<td>II</td>
<td>T1N0M0</td>
<td>75</td>
<td>+</td>
</tr>
<tr>
<td>EVSCC7PX</td>
<td>TG</td>
<td>19</td>
<td>M</td>
<td>IV</td>
<td>T2N0M0</td>
<td>None</td>
<td>+</td>
</tr>
<tr>
<td>EVSCC8PX</td>
<td>TG</td>
<td>62</td>
<td>F</td>
<td>II</td>
<td>T2N0M0</td>
<td>30</td>
<td>ND</td>
</tr>
<tr>
<td>EVSCC9PX</td>
<td>TG</td>
<td>65</td>
<td>M</td>
<td>IV</td>
<td>T4N1M0</td>
<td>40</td>
<td>ND</td>
</tr>
<tr>
<td>EVSCC10M</td>
<td>HPX</td>
<td>66</td>
<td>M</td>
<td>IV</td>
<td>T4N1M0</td>
<td>50</td>
<td>+</td>
</tr>
<tr>
<td>EVSCC11M</td>
<td>BC</td>
<td>66</td>
<td>M</td>
<td>IV</td>
<td>T3N2aM0</td>
<td>45</td>
<td>ND</td>
</tr>
<tr>
<td>EVSCC12N</td>
<td>P</td>
<td>69</td>
<td>M</td>
<td>I</td>
<td>T4N1M0</td>
<td>50</td>
<td>ND</td>
</tr>
<tr>
<td>M</td>
<td>HPX</td>
<td>52</td>
<td>M</td>
<td>I</td>
<td>T2N2cM0</td>
<td>90</td>
<td>ND</td>
</tr>
<tr>
<td>EVSCC13N</td>
<td>Lx</td>
<td>59</td>
<td>M</td>
<td>IV</td>
<td>T2N2cM0</td>
<td>45</td>
<td>ND</td>
</tr>
<tr>
<td>M</td>
<td>TX</td>
<td>56</td>
<td>M</td>
<td>III</td>
<td>T3N0M0</td>
<td>53</td>
<td>ND</td>
</tr>
<tr>
<td>EVSCC14M</td>
<td>TL</td>
<td>55</td>
<td>M</td>
<td>III</td>
<td>T1N0M0</td>
<td>70</td>
<td>+</td>
</tr>
<tr>
<td>EVSCC15P</td>
<td>TX</td>
<td>34</td>
<td>M</td>
<td>I</td>
<td>T4M0M0</td>
<td>None</td>
<td>+</td>
</tr>
</tbody>
</table>

*a Tumor sites: tonsil (TL), hypopharynx (HPX), larynx (TG), tongue (TG), buccal cavity (BC).
*b PX suffix designates primary tumor grown as nude mouse xenograft; N, P, M are normal tissue, primary tumor, or metastasis, respectively.

Fig. 1. Immunocytochemical staining of p53 in SCCHN. a, positive nuclear staining of FaDu cells; b, negative staining of SiHa cells; c, positive cytoplasmic staining of EVSCC1 cells; d, positive nuclear staining of UMSSCC10B cells; e, positive nuclear staining of fresh tumor sample EVSCC5PX. Cells were reacted with PAbl801 in a, b, d, and e, and with PAb240 in c.

with the predicted chain termination and loss of nuclear localization signals in the carboxyl terminus of the p53 protein (19).

UMSCC19 had a 10-base pair deletion that resulted in the deletion of codons 248 to 250 inclusive, and a frame shift involving codon 251. Tumor xenograft EVSCC4X had a 2-base pair (TT) addition at codon 289–290 resulting in a frame shift. Only the mutant allele was found in 6 of the SCCHN cell lines, the 2 xenografts, and the 2 xenografts, indicating that the p53 locus was hemizygous or homozygous in these cells. This finding implies that the wild-type allele is either transcriptionally inactive or has been lost by the tumor cells. Both mutant and wild-type p53 alleles were detected in UMSCC23 and UMSCC63, indicating that either the normal allele was retained in the tumor cells or the cell lines are a mixture of tumor and normal cells. The latter explanation is unlikely since immunostaining with p53 antibodies showed uniform nuclear staining of all UMSCC23 and UMSCC63 cells.

Detection of p53 mutations in SCCHN cell lines prompted us to investigate the prevalence of aberrant p53 in primary explant cultures of tumors either resected directly from the patient or propagated as xenografts in nude mice. We evaluated tumor tissue from 13 patients for increased p53 protein by immunocytochemical staining with PAb 1801 and PAb 240. In two cases, normal tissue adjacent to the tumor and, in four cases,
lumph node metastasis from the patient were evaluated in parallel with the primary tumor. Tumor tissue from 10 (77%) of 13 patients exhibited increased p53 protein (Table 2; Fig. 1e). This is a minimal estimate since not all primary explant cultures were stained with both PAb 1801 and PAb 240. An intriguing observation is that cells from four of the five metastases were positive and, in one of these cases (EVSCC13), both the normal tissue and primary tumor were negative for p53 expression. Although the numbers are small, the data suggest that p53 overexpression may be an important marker in tumor progression. In a separate analysis of p53 gene mutations in 5 primary tumors, 2 were mutated for p53 at codons 175 and 193. Both were single base pair mutations which resulted in alterations in codon usage (data not shown). Together these data encourage the analysis of a larger number of SCCHN.

In this study we have demonstrated that p53 mutations can be detected at a high frequency in SCCHN. This finding confirms two recent reports of elevated p53 expression in SCCHN (20, 21), and provides strong support for the view that p53 gene mutations are likely to play an important role in the pathogenesis of head and neck cancer. A striking finding from our work is that G to T transversions are detected in the majority of SCCHN cell lines and that codons 245 and 248 may represent mutational hot spots in head and neck cancer. G to T transversions are the most frequent p53 mutations in non-small cell lung cancer (3, 4, 22) and are found in four of the four esophageal squamous cell carcinomas (5, 6), two other tobacco-related cancers. It is worth noting that benzo(a)pyrene, a major carcinogen in tobacco smoke, is known to induce G to T transversions (23, 24). Thus, our data link tobacco use, a known risk factor in head and neck cancer, with G to T transversions in p53 and suggest that these mutations may be footprints of tobacco carcinogens.

5 G. Casey, unpublished data.

ACKNOWLEDGMENTS

The authors thank Dr. Tom Carey for providing cell lines and gratefully acknowledge the help of Mary Richardson in preparing the manuscript.

REFERENCES

10. Limon, J., Da Cm, P., and Sandberg, A. A. Application of long-term colla
22. Suzuki, H., Takahashi, T., Kuroishi, M., Suyama, M., Ariyoshi, Y., Taka
Frequent \textit{p53} Mutations in Head and Neck Cancer

Kenneth D. Somers, M. Anne Merrick, Martha E. Lopez, et al.