Expression of Mutated p53 Occurs in Tumor-distant Epithelia of Head and Neck Cancer Patients: A Possible Molecular Basis for the Development of Multiple Tumors

Matthias Nees, Nils Homann, Hilde Discher, Thomas Andl, Christel Enders, Christel Herold-Mende, Antje Schuhmann, and Franz X. Bosch

Molecular Biology Laboratory, Ear, Nose, and Throat University Hospital, 69120 Heidelberg, Germany

ABSTRACT

As in most other tumor types, expression of mutated or phenotypically altered p53 is a common occurrence in head and neck carcinogenesis. Since the prognosis for many head and neck tumor patients is severely affected by the occurrence of multiple primary and secondary tumors, we have analyzed the phenotype and genotype of p53 in squamous and respiratory epithelia either adjacent to or at significant distance from the primary tumors. Many tumor patients showed multifocal overexpression of the p53 protein in a variety of these epithelia. Overexpression of p53 correlated with increased proliferation and dedifferentiation, as demonstrated by immunohistochemistry and in situ hybridization using histone H3 and cytokeratin-specific probes. Polymerase chain reaction-single-strand conformation polymorphism analysis and sequencing of p53 DNA, amplified from these biopsies after immunostaining and microdissection, confirmed and extended these findings. We have identified different mutations in p53 in different tumor-distant epithelia from the same patients. The data indicate that mutation of p53 is an early event in head and neck carcinogenesis, preceding signs of overt neoplasia, and that different mutations in p53 in multiple foci may provide a molecular basis for the development of multiple tumors.

INTRODUCTION

Carcinogenesis of the head and neck is marked by the involvement of carcinogenic factors derived from tobacco and alcohol abuse, as well as from nutritional, environmental, and occupational exposure (1). Head and neck tumors, consisting mostly of SCC, show a highly variable distribution in different parts of the world (2) and are among the most aggressive human malignancies, with a high incidence of metastasis. The prognosis for patients suffering from head and neck cancers is even more adversely affected by the very high occurrence of multiple primary and secondary tumors (3), a phenomenon which among clinicians is referred to as field cancerization.

The sequence of genetic changes underlying the development and progression of head and neck cancer is not known. However, as in most other tumor types (for reviews, see Refs. 4-9; for reviews, see Refs. 7-9 and references cited therein). The p53 protein level strongly increases after experimental DNA damage, and this is followed by a specific arrest of the cell cycle in G1 (14-16). Cells containing mutated p53 or lacking p53 are unable to induce this cell cycle arrest (17, 18). Furthermore, they have a reduced capacity to induce apoptosis after DNA damage by ionizing radiation or some therapeutic drugs (19, 20).

This current concept of p53 as a key cell cycle checkpoint and "guardian" against genetic damage (7, 21) and the recent discovery that genetic damage, i.e., genomic instability, precedes overt tumorogenesis (Refs. 22-24; see, however, Refs. 25 and 26) are further supported by a number of recent publications. p53 alterations occur in cancer-prone diseases and precancerous lesions in various body sites including the lung (27, 28), the esophagus (29), the brain (30), and the colon and rectum (31). In the head and neck region, mutations in the p53 gene have thus far been identified mainly in SCC cell lines (32-34) and in only a limited number of primary tumors (33, 35). Overexpression of p53 protein, as detected by immunohistochemistry, has also been observed in hyperplastic and dysplastic epithelia adjacent to the tumors (36-38).

These recent developments have prompted us to ask whether expression of aberrant p53 might occur early in head and neck carcinogenesis as a possible molecular basis for the frequent and very severe problem of field cancerization. Our studies included the parallel assessment of proliferation and differentiation in the biopsies examined, to determine whether there is a functional relationship between the biological behavior of the tissues and their p53 status. The results strongly indicate that aberrant p53 indeed occurs very early, being detectable in epithelia at a considerable distance from the primary tumors. At both the protein and the DNA levels, we have observed multifocal and discontinuous changes in p53, providing the molecular basis for a multifocal development of multiple tumors.

MATERIALS AND METHODS

Tissue Specimens. Specimens from different types of head and neck tumors were collected in the operating room immediately after operation. All specimens of squamous cell carcinomas showed, at least in some areas, signs of dedifferentiation, with histological grading varying between G2 and G4. With the consent of some patients undergoing radical neck dissection, hyperplastic and dysplastic squamous and respiratory epithelia in close vicinity to, as well as at various distances from, the tumor were also obtained and were processed separately. The tissues were snap-frozen in isopentane/liquid nitrogen and stored at -80°C. Serial cryostat sections (5-6-μm thick) were mounted on 3-aminopropyltriethoxysilane-coated slides.

IHC. The frozen sections were fixed for 10 min in acetone at -20°C and air dried. Primary monoclonal antibodies were applied to the sections in phosphate-buffered saline, pH 7.4. Antibody staining, performed on at least two sections per specimen, was detected by the alkaline phosphatase-anti-alkaline phosphatase method, using the protocol provided by Dakopatts (Copenhagen, Denmark).
Antibodies. Three different Mabs directed against p53 were used. Bp5311 (39) (commercially available from Progen Biotechnics, Heidelberg, Germany) is a newly developed, highly sensitive antibody recognizing an antigenic determinant in the amino-terminal region of the p53 protein and reacting with both wild-type and mutant forms of p53.4 DO 7 (40) is also a pan-reactive monoclonal antibody, whereas PAb 240 (41) reacts only with mutated p53 protein. These were obtained from Medac (Hamburg, Germany). The cytokeratin-specific Mabs used were K, 17.2, K, 18.174, and K, 19.1, reacting with cytokeratins 8, 18, and 19, respectively, and 6B10 and IC7, specific for cytokeratins 4 and 13, respectively (for references and sources, see Ref. 41).

ISH and Complementary DNA Probes. The procedure was carried out as described previously (41-44). The antisense complementary RNA hybridization probes were labeled by in vitro transcription from Bluescript-based vectors with either [α-32P]CTP or [γ-32P]UTP, to a specific activity of approximately 5 x 106 to 107 cpm/μg RNA. The hybridization solution contained 50 mM dithiothreitol, and the sections were covered with Parafilm to avoid evaporation of dithiothreitol. The RNase A (10 μg/ml) treatment after hybridization was done for 30 min at 37°C. At least two sections were hybridized with each probe. The Bluescript-based human histone H3 complementary DNA, containing a 330-base pair insert, was a kind gift of Drs. H. Smola and N. E. Fusenig, German Cancer Research Center (Heidelberg, Germany). The complementary DNA probes used to analyze mRNA expression patterns of cytokeratins have been described previously (41, 42) and were kind gifts of Drs. B. Bader, R. Leube, and W. W. Franke, also of the German Cancer Research Center.

Microdissection from Frozen Sections and PCR Amplification. Frozen sections were stained with Bp53-11 and Pab 240 and air-dried. Small areas showing various reactivities, from zero to largely positive, were scraped off under the light microscope using a fine needle. We did not attempt to quantitate accurately the numbers of epithelial cell nuclei prepared for PCR in this fashion, which varied between approximately 5 and 50. Instead, we aimed at the greatest possible enrichment of these nuclei. Contamination with cells in the connective tissue was, however, unavoidable, especially in the smallest preparations. DNA was prepared from the scraped-off material by digestion with 100 μg/ml proteinase K for 120 min at 56°C. After inactivation of proteinase K at 80°C for 10 min, the DNA was amplified by PCR. Negative controls for PCR amplifications were derived from the proteinase K buffer alone and from material obtained from microdissection of sections which had been extensively UV-cross-linked. In these cases no amplified product was detected. In the first PCR reaction, a 1647-base pair fragment encompassing exons 5 to 8 was produced. The products of this first PCR were the source for two separate second PCRs; first, individual exons were reamplified for SSCP analysis.

PCR-SSCP Analysis. The method was based on the studies of Orita et al. (45). The products of the second PCR reaction were radioactively labeled either by direct labeling of both DNA strands in the presence of [α-32P]dCTP in the reaction mixture or by labeling of either one of the two DNA strands by using one 5'-end-labeled primer (using [γ-32P]dCTP and polynucleotide kinase). The PCR products were denatured at 85°C for 3 min in 50% formamide buffered with 20 mM Tris-HCl, pH 8.0, and were quenched in ice-water. Aliquots were run on native 6% polyacrylamide gels, with an acrylamide to bisacrylamide ratio of 99:1, containing 10% glycerol. Electrophoresis was either by direct labeling of both DNA strands in the presence of [α-32P]dCTP or 35S-UTP, to a specific activity of approximately 5 x 108 cpm/μg DNA.

Bluescript-based human histone H3 complementary DNA, containing a 330-base pair insert, was a kind gift of Drs. H. Smola and N. E. Fusenig, German Cancer Research Center (Heidelberg, Germany). The complementary DNA probes used to analyze mRNA expression patterns of cytokeratins have been described previously (41, 42) and were kind gifts of Drs. B. Bader, R. Leube, and W. W. Franke, also of the German Cancer Research Center.

RESULTS

Screening of Tumors for Overexpression of p53 Protein by Immunohistochemistry. A total of approximately 170 biopsies from the head and neck region, ranging from apparently normal mucosa to lymph node and skin metastases, were immunohistochemically stained on cryostat sections for overexpression of p53 protein. Only biopsies of primary SCC of different histological grades were included in this study. The great majority (96%) of the patients had a history of smoking and drinking. Squamous epithelia obtained from surgery of non-tumor-bearing patients served as negative controls. These never reacted positively with any p53 antibody. All tumor sections were routinely tested with antibodies Bp53-11, DO 7, and Pab 240, of which Bp53-11 in our hands was the most sensitive anti-p53 antibody. All histologically conspicuous mucosal biopsies positive with Bp53-11 were also tested with Pab 240, while normal-appearing epithelia negative with Bp53-11 were not. Table 1 summarizes the results of these staining series on the SCC biopsies. The tumors were grouped according to their anatomical site, and it appears that the percentage of p53-immunoreactive tumors is highest in the oropharynx (80%) and oral cavity (67%), decreasing somewhat in the regions of larynx (44%) and hypopharynx (38%). Interestingly, in a few cases we have observed differential p53 immunoreactivity between the primary tumor and the lymph node metastasis (cases 3 and 9 of Table 2 and others not included in Table 2).

<table>
<thead>
<tr>
<th>Tumor Sitea</th>
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<th>+</th>
<th>-</th>
<th>S+</th>
<th>S-</th>
<th>NS+</th>
<th>NS-</th>
<th>%</th>
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<td>1</td>
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<td>6</td>
<td>10</td>
<td>5</td>
<td>8</td>
<td>3</td>
<td></td>
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</tr>
<tr>
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<td>5</td>
<td>1</td>
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<td>1</td>
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</tr>
</tbody>
</table>

a Tumor sites: LAR, larynx; HPX, hypopharynx; OPX, oropharynx; OCA, oral cavity; TNS, tonsils. b n, total number; +, number of p53-positive cases; -, number of p53-negative cases; S, smoker; NS, nonsmoker; ?, unknown smoker status.
within single biopsies. Positive staining in tumor-distant biopsies was
also seen in patients with immunonegative primary tumors, most
notably in patient 3 of Table 2, in whom the primary tumor did not
react with any antibody but the tumor-adjacent epithelium, the lymph
node metastasis, and one tumor-distant site did react positively. We
observed differential staining between primary tumors and lymph
node metastases in several instances (see also patient 9 of Table 2). A
similar observation has been made by Somers et al. (34) but not by
Dolcetti et al. (38). It must be stressed that we have also seen severely
dysplastic but nonreactive regions both in the direct vicinity of im-
munopositive tumors (see case 6 in Table 2) and in epithelia distant
from immunonegative primary tumors. It was interesting to note that
patients with N0 tumors (no diagnosed regional lymph node meta-
stases; patients 4, 5, 6, 10, 14, and 15) showed similar multifocal over-
expression of p53.

Relationship of Overexpression of p53 in SCC and Tumor-
distant Epithelia to the Proliferation and Differentiation Status.
Overexpression of the p53 protein in nontumorous cells could reflect
cell cycle arrest due to genetic damage. In this case, the cells over-
expressing p53 would not be proliferating. Alternatively, overexpres-
sion could be the result of functional inactivation of the p53 protein
itself and would reflect inactivation of cell cycle control. In this case
(as in the tumors) the cells overexpressing p53 would actively prolif-
erate and show a change towards neoplasia. To clarify the meaning of
the p53 positivity in the tumor-distant epithelia, we selected the largest
of these biopsies to analyze the expression of the histone H3 gene at
the mRNA level (by ISH), as a marker for proliferation. In addition,
the differentiation status was assessed at both the protein level (by
IHC) and the mRNA level (by ISH), by analysis of the expression
profile of the ck gene family. As a representative experiment, Fig. 1
presents the expression patterns of the p53 protein, histone H3 mRNA,
and specific ck proteins or mRNA in a metastasizing SCC of the
epiglottis (left column; this is case 7 of Table 2) and in the very mildly
dysplastic mucosa of the vocal cord of a patient with a N0 tumor of the
larynx (right column; this is case 6 of Table 2). It can be seen that the
p53-positive cells in the invisibly growing epiglottis tumor (Fig. 1a,
showing red alkaline phosphatase-anti-alkaline phosphatase-stained
nuclei) strongly express histone H3 mRNA (Fig. 1b, bright spots upon
dark-field illumination). The invasive cells also strongly express the
simple epithelial-type ck 8 and ck 18 (Fig. 1, c and d, red cytoplasmic
staining). The adjacent nondysplastic epithelium showed weak and
heterogeneous cytokeratin 8 staining and no cytokeratin 18 staining
(Fig. 1, c and d, arrowheads). In the early dysplastic foci of the
tumor-distant vocal cord mucosa (Fig. 1e), only very few cell nuclei
were distinctly p53 positive (Fig. 1e, arrowheads); some others were
stained only faintly or were negative (Fig. 1e, open arrow). Most of
these basal cells showed strong expression of histone H3 (Fig. 1f,
arrowheads), indicating that they were actively proliferating. The
same area in another consecutive section showed, at the protein level,
a diffuse and heterogeneous induction of ck 8 (Fig. 1g). However, at
the mRNA level, as analyzed by in situ hybridization, the entire early
dysplastic area showed a strong induction of the ck 8 gene in basal and
parabasal cells (Fig. 1f), at a level similar to that of invasive tumor
cells (data not shown). Only very weak or no induction of cytokeratin
18 was observed (Fig. 1h).

Genetic Analysis by PCR-SSCP of Genomic p53 DNA from the
Central Hot Spot Region (Exons 5–8). Upon assessment of prolif-
eration and differentiation, the vast majority of SCC of the head and
neck show an extensive biological heterogeneity, both at the histo-
logical level and at the molecular level. This heterogeneity also holds
t true for the p53 antigen, since within a short distance on the same
frozen section immunoreactive and nonreactive areas can be found
both in the tumors (data not shown) and in the tumor-surrounding
epithelia (Fig. 1e). In addition, tumor and mucosal biopsies contain
highly variable amounts of stroma and/or lymphoid tissue. This makes
the use of whole biopsies as a source of genomic DNA for amplifi-
cation by PCR virtually impossible. Rather than using unstained sec-
tions (27) or hematoxylin-eosin-stained sections (28, 29) for micro-
dissection of material with different gene expression patterns, we
proceeded to use sections which were stained with p53-specific anti-
odies immediately before microdissection. This was a prerequisite to
directly compare, e.g., in single tumor islands or small areas within
epithelia, the immunohistochemical reactivity with the genetic status
of p53. PCR-SSCP is suitable for screening large numbers of lesions
for specific genetic alterations (45) and, in the case of p53, attempting
to correlate the immunohistochemical screening showing overexpres-
sion of the p53 protein with the presence of mutations within the p53
gene.

The PCR-SSCP technique was set up by analyzing a small number
of immunopositive and immunonegative tumors with known and un-
known p53 mutations. We used direct labeling of the PCR products or,
alternatively, employed only one end-labeled primer. In this manner,
we obtained reproducible SSCP results from separate analyses of the
same tumor, with sequencing confirming the identity of the mutation.
The SSCP patterns were, however, readily interpretable only for exons
5, 7, and 8, whereas analysis of exon 6 yielded additional bands under
all conditions, due to a variable number of alternative conformations.

We then analyzed exons 5, 7, and 8 in tumor-adjacent and tumor-
distant epithelia of diverse but always positive immunoreactivity.
Different microdissected regions from the biopsies were analyzed to
Fig. 1. Expression of p53, histone H3, and simple epithelial cytokeratins in primary tumor and tumor-distant mucosa. a-d, protein expression patterns of p53 (a), ck 8 (c), and ck 18 (d) and H3 mRNA expression pattern (b, dark-field illumination, with positive signals as bright spots) in an epiglottis tumor (T₂N₂M₀, G₂). c–f, analogous patterns in the mucosa of the vocal cord from a patient with a larynx tumor (T₂N₂M₀, G₂). In addition, the mRNA levels of ck 8 are shown in i (dark-field photomicrograph). In a and b (parallel sections) only invasive tumor islands are present. Arrowheads in c and d (parallel sections), nondysplastic epithelium directly adjacent to invasive tumor islands.
of cells overexpressing p53 and expressing H3 mRNA; open arrows, an area with weakly p53-positive cells which also express H3 mRNA. Note that most but not all p53-overexpressing cells also express H3 mRNA (a, b, c, and f). Note disparate expression of ck 8 and ck 18 in the tumor-distant epithelium (compare g and h with c and d), similar to the squamous epithelium directly adjacent to invasive tumor cells in c and d (arrowheads). Note the strong induction of ck 8 mRNA in the entirely mildly dysplastic area (i).
eral cells in SCC. This conclusion is based on the consistent obser-

change to a more dedifferentiated state, again similarly to the periph-

ary cell cycle arrest but rather a loss of cell cycle control. That overexpression of p53 in tumor-distant cells reflected not a tem-

porary but rather a loss of cell cycle control. The analysis provides an excellent sensitive marker of proliferative activ-

tion. In dysplastic and neoplastic lesions, H3 mRNA levels were

significantly elevated levels only in areas with connective tissue inflamma-

tion. These areas were associated with strong expression of the histone H3

expression is tightly regulated within the cell cycle at the

p53-positive cells formed the tips of early, mildly dysplastic lesions

of these epithelia were histologically inconspicuous but frequently the

must reside in other regions of the p53 gene. Different p53 mutations

do not only point mutations but included a deletion of 41 bases and

an insertion of a dinucleotide, disrupting the reading frame of the p53

must reside in other regions of the p53 gene. Different p53 mutations

during tumor-distant biopsies were also found in other patients

and in different exons, confirming the respective SSCP results. These

were not only point mutations but included a deletion of 41 bases and

an insertion of a dinucleotide, disrupting the reading frame of the p53

mRNA (data not shown). Sequence analysis of many microdissected

regions from tumor-distant epithelia is in progress.

**DISCUSSION**

The work presented is of relevance to the most severe problem

affecting prognosis and management of head and neck cancer patients,

i.e., the occurrence of multiple primary, secondary, and recurrent

tumors (also referred to as field cancerization). In a surprisingly large

number of tumor-distant mucosal biopsies, mostly not exclusively

from patients with p53-immunopositive primary tumors, we detected

cells or groups of cells overexpressing the p53 protein (Table 2). Some

of these epithelia were histologically inconspicuous but frequently the

p53-positive cells formed the tips of early, mildly dysplastic lesions

(Fig. 1). In all cases examined, the positive p53 immunoreactivity in

these areas was associated with strong expression of the histone H3

gene. H3 expression is tightly regulated within the cell cycle at the

mRNA level, rising to peak levels in late G₂/early S phase and very

rapidly declining in late S phase. In epithelial lesions of the head and

neck, H3 mRNA levels, as detected by ISH, were very low in normal

squamous epithelia of the upper aerodigestive tract (42, 43, 46–48).

They are, however, consistently detected in squamous cell carcinomas

(46, 47). Although a systematic analysis is still lacking, there is

evidence from several studies on natural as well as experimentally

induced malignant and premalignant epithelial lesions that neoplastic

and dysplastic changes, but not reversible pathological processes like

gingivitis, are associated with a neo-expression of these simple epi-

thelial-type cytokeratins (41, 48, 49). It is specifically noteworthy in

the context of this work that, after injection or transplantation of

immortalized but nontumorigenic human keratinocytes into nude

mice, the expression of ck 8 and/or 18 occurred only in the prolifera-
tive phase of these cells. After growth arrest, expression of these

cytokeratins was down-regulated, whereas expression of ck 19 and ck

1 continued (43). We therefore conclude that the p53 immunopos-

itivity, together with the expression of the histone H3 gene and of at least

one of the simple epithelial cytokeratins, represents a significant

change of these cells towards neoplasia.

It will be interesting to determine whether expression of the simple

epithelial-type cytokeratins, which can be easily monitored by immu-

nohistochemistry using a panel of highly sensitive and specific mono-

clonal antibodies, can serve as an independent marker for the process

of multiple tumor development. This would be useful in those cases in

which mutation of p53 is not involved or is not recognized by p53-

specific antibodies.

The analysis of the genetic status of the p53 gene by PCR-SSCP and

PCR-sequencing has confirmed the validity of our concept that the

phenotypic changes observed as early events in head and neck tumor-

 genesis, being detectable even in histologically inconspicuous epi-

thelia at significant distance from the primary tumor, were indeed due

to p53 mutations. Single-strand conformation polymorphisms were

observed with similar frequency, compared with the phenotypic

**Table 3 Selected informative SSCP results on exons 5, 7, and 8 in epithelia surrounding head and neck tumors**

<table>
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<th>Patient, tumor</th>
<th>Mucosal biopsy*</th>
<th>Exon 5 direct</th>
<th>Exon 7 direct</th>
<th>Exon 8 direct</th>
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<tr>
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<td>wt</td>
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* EES, entrance to esophagus; LPH, lateral pharynx wall; SGL, subglottis. For all other localizations, see footnotes to Table 2.
changes observed by gene expression analysis. In fact, the SSCP findings are particularly striking because we have clearly detected different genetic changes in p53 in different tumor-distant mucosal biopsies from the same patients. Dramatic changes occurred even in different regions within a single biopsy (Table 3). These experiments have revealed extensive biological and genetic heterogeneity within the tumor-distant epithelia, and some of the single-strand band shifts were probably derived from small subpopulations of cells. Evidently, such heterogeneity may be an obstacle to the unambiguous identification of the mutations present. We therefore selected biopsies with moderate electrophoretic changes in exons 5 (Fig. 3) and 7 (data not shown) to see whether we could detect the mutations indicated by the SSCP analysis. As demonstrated in Fig. 3a, we could clearly identify in the sequence derived from the lateral pharynx wall of patient 3 an additional thymidine band and the concomitant decrease in the guanosine band in codon 176 (TGC to TTG, resulting in a amino acid change from cysteine to leucine), indicating either a heterozygous state or approximately the same number of cells being wild-type and homozygous mutant. We are also confident that we have detected in a minor subpopulation of cells from the base of the tongue, obtained also from patient 3, a guanosine to adenosine transition, resulting in a change from GAT/aspartate to AAT/asparagine in codon 148 (Fig. 3b). Regarding the possibility of sequence errors introduced into two wild-type alleles by the Taq polymerase, one must keep in mind that such an error would have to occur in the very first cycle to reach a maximum of 25% base substitution. For this reason and because these genetic changes were found in p53-immunopositive areas, we can rule out this possibility.

Of the 15 patients represented in Table 2, five have developed secondary or recurrent tumors within the last 2 years (approximately 30%), two of these have died from multiple secondary tumors (patients 2 and 3), and one has died from distant metastasis. Our data are of relevance to several aspects of the molecular and cellular changes occurring during head and neck tumorigenesis. (a) Together with recent reports on p53 mutations in other organs (for references, see “Introduction”), the results indicate that p53 mutations in many cases are early events in tumorigenesis. (b) It has been proposed that the process of multiple tumor development in the head and neck region might be initiated by lateral movement of premalignant basal keratinocytes, thus favoring a monoclonal nature for the development of multiple primary, secondary, and recurrent tumors (37). However, our analyses of tumor-distant biopsies (where field carcinization begins) show a markedly discontinuous and multifocal topography of the cells expressing aberrant p53, on both both the antigen level (IHC) and the genetic level (SSCP and DNA sequencing), and therefore strongly argue for a multifocal polyclonal process. This concept is strongly substantiated by the identification of different p53 mutations in primary and second primary tumors (50). The identification of the mutations persisting from the tumor-distant mucosa to primary and secondary tumors will prove this point. These efforts should also yield information on whether the different dysplastic foci with mutated p53 progress by clonal expansion, as proposed by Nowell (51) and Fidler and Kripke (52) and as recently substantiated for brain tumors (53). (c) If the current concept that normal p53 is a key cell cycle checkpoint, arresting cells in G1, to allow repair of genetic damage, is correct, one should expect genetic instability of the tumor-distant cells harboring mutated p53 (also discussed in Ref. 9). Interphase cytogenetic studies on structural and numerical chromosomal aberrations in tumor-surrounding epithelia should be useful to address this question. (d) Regarding the multifactorial nature of carcinogenesis, it seems likely from our data that other genetic changes occur in the tumor-distant mucosa and contribute to and accelerate the progression of these lesions to solid tumors. At present, there is no information as to which other tumor suppressor genes might be involved. It is to be hoped that recognizing mutations in tumor-surrounding biopsy specimens and thereby identifying tumor patients at high risk of developing additional tumors will eventually improve the prognosis for these patients.

Fig. 3. DNA sequence comparison of exon 5 from microdissected regions of the base of the tongue of patient 3 (left) and a region of the lateral pharynx wall also of patient 3 (right) (Fig. 2A, top lane 7 versus lane 6). a, sequences around codon 176. Upper dot, additional thymidine band in cells from the lateral pharynx wall (note also the decrease in the adjacent guanosine band); arrowhead and lower dot, corresponding single wild-type thymidine band in cells from the base of the tongue. b, sequences around codon 148. Arrowhead, mutation in codon 148 in a subpopulation of cells from the base of the tongue region; dots, corresponding position in the wild-type sequence of the lateral pharynx wall.
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Expression of Mutated p53 Occurs in Tumor-distant Epithelia of Head and Neck Cancer Patients: A Possible Molecular Basis for the Development of Multiple Tumors

Matthias Nees, Nils Homann, Hilde Discher, et al.


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