

Cancer of the Tongue in Patients Younger Than 40 Years

A Distinct Entity?

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Background: An increase in the incidence of oral cancer among patients younger than 40 years has been reported worldwide. It has been suggested that the disease behaves more aggressively among young people.

Objective: To evaluate the clinical and biologic behavior of tongue cancer among younger patients.

Patients and Methods: Data on all patients younger than 40 years in whom tongue cancer was diagnosed in Finland between 1980 and 1989 (34 cases) were collected; pertinent clinical data were available for risk factor screening. A follow-up of at least 5 years or until death was available for all 34 patients. Immunocytochemical staining techniques were used to assess the expression of p53 and bcl-2 proteins, and p53 mutation analysis was performed by using the nonradioactive single-strand conformation polymorphism technique.

Results: The incidence of tongue cancer in this age group in Finland did not change during the study period. The clinical behavior of tongue cancer in young people was not more aggressive compared with

that of older patients in general, with the overall 5-year survival being as good as 70.6%. Altogether, p53 mutations were found in 17 of 33 tumors (51.5%). The p53 and bcl-2 protein expression was strong or moderate in 33.3% and 30.3% of the samples, respectively. Intense p53 protein expression was associated with the larger tumor size ($P<.05$). The poorest prognosis was found in patients with tumors greater than 4 cm in diameter ($P=.01$) or moderately or poorly differentiated cancer ($P=.01$). There was a trend for the adverse prognosis to accumulate in patients with moderately or poorly differentiated carcinoma and mutations in p53 ($P=.09$).

Conclusions: The cause of tongue cancer in patients younger than 40 years seems to be multifactorial. Those patients had a similar clinical course, prognosis, and function of p53 as found in the reports of a normal age variation. Mutations of p53 seemed to be an additional prognostic marker that was associated with moderate or poor differentiation of the tumors.

Arch Otolaryngol Head Neck Surg. 1996;122:1313-1319

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EVIDENCE HAS accumulated that implicates an increase in the incidence of oral cancer among patients younger than 40 years worldwide. Oral cancer is considered to be primarily a disease that occurs in men (in their 60s) who have been smokers, with younger patients accounting for less than 4% of all oral cancers. Recently, however, reports on the increasing incidence and mortality rates of oral cancer among younger people have accumulated from several European countries¹ and other continents as well.² Unlike the older patients, many of these younger patients have never smoked or consumed alcohol excessively.³ Furthermore, in these patients, the exposure to carcinogens might be of a duration that is too short for malignant transformation,⁴ but other causal factors for the

early onset of disease have not been disclosed as yet.

Some authors regard the causal factors⁵ and the clinical course of the disease⁶ to be identical to that found in older patients. However, other investigators consider tongue cancer as a more aggressive disease among younger patients; despite the early diagnosis and proper clinical management, a poor response to therapy and a dismal outlook are common.⁷

The suggested more malignant behavior of oral cancer among younger patients has raised the concept that it may be a distinct entity, differing from that of

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PATIENTS AND METHODS

The participants in this study consisted of all patients with a squamous cell carcinoma of the tongue that was diagnosed in Finland (population, 5.1 million) between January 1980 and December 1989; these patients were younger than 40 years at the time of diagnosis. From the files of the Finnish National Cancer Registry, a total of 34 patients were found. The clinical data on these patients were collected from their medical records, and paraffin-embedded biopsy specimens that were taken prior to any therapy were collected for this analysis. All but 1 original paraffin-embedded block were available. **Table 1** and **Table 2** summarize the detailed clinical data with regard to the patients.

CLINICAL DATA

Data on the smoking habits and alcohol consumption were available for 24 and 18 patients, respectively. The smokers were divided into 3 groups: (1) nonsmokers and those who had quit smoking more than 5 years previously ($n=9$); (2) moderate smokers (those who had smoked less than 20 cigarettes daily or had smoked for less than 5 years or had quit smoking less than 5 years ago) ($n=4$); and (3) heavy smokers (those who had smoked more than 20 cigarettes daily and had smoked for more than 5 years) ($n=11$). Alcohol consumption of the patients was graded as social or heavy; there were 16 social and 2 heavy consumers.

The patients had been treated at 14 different hospitals in Finland. All but 1 patient who was suffering from Fanconi anemia and whose squamous cell cancer was inoperable underwent a hemiglossectomy or an otherwise radical resection of the tumor. Neck dissection, either radical, functional, or supraomohyoidal, was carried out in 18

patients (53%). Preoperative radiotherapy to the tumor and neck was given to 4 patients, and 17 patients received postoperative radiotherapy. Both preoperative and postoperative radiotherapy was given to 3 patients. Chemotherapy was given preoperatively and postoperatively to 2 and 3 patients, respectively.

Five-year follow-up data were available for all patients; 10-year follow-up data or data until death were available for 18 patients (Table 1).

HISTOLOGIC STUDIES

Hematoxylin-eosin-stained sections were made from all biopsy specimens to confirm the diagnosis of a squamous cell carcinoma. The samples were divided into well-, moderately, and poorly differentiated carcinomas.

IMMUNOCYTOCHEMICAL STAINING METHODS FOR p53 AND bcl-2 PROTEINS

Sections (5 μ m thick) on 2% 3-aminopropyl-triethoxysilane-coated (Sigma Chemical Co, St Louis, Mo) slides were deparaffinized and dehydrated through a series of xylene and graded alcohol. For *bcl-2* staining, the sections were treated in a microwave oven in citric acid buffer (pH, 6.0) for 5 minutes to intensify the antibody reaction. Endogenous peroxidase was blocked by using 5% hydrogen peroxide in phosphate-buffered saline solution for 5 minutes. For *p53* staining, the samples were incubated in normal goat serum (Vectastain ABC kit, Vector Laboratories, Inc, Burlingame, Calif) for 15 minutes, followed by incubation with rabbit polyclonal antibody for *p53* (dilution, 1:1000) (CM1, Novocastra Laboratories Ltd, Newcastle upon Tyne, England) at 4°C overnight. For *bcl-2* staining, the sections were incubated in normal horse serum (Vectastain ABC kit), followed by incubation in *bcl-2* primary monoclonal

the older patients.⁸ In addition to the minor importance of the exogenous factors, the biologic and genetic mechanisms behind the disease in younger and older patients may be different.

The *p53* tumor suppressor gene is responsible for the G₁-phase arrest in the cell cycle following a genotoxic damage, thus allowing the cell to repair the DNA defect before the next division. It also participates in the commitment to apoptosis after cellular or genetic damage.⁹ Environmental factors (eg, cigarette smoking) are known to cause *p53* mutations.¹⁰ Alternatively, the *p53* protein function can be blocked by binding with some viral oncoproteins, including the E6 protein of the human papillomavirus types 16 and 18.¹¹ The *p53* mutations and overexpression of *p53* protein are detected in more than 50% of oral malignant neoplasms¹²; thus, these constitute the single most common genetic defect in these cancers. The *p53* mutations have also been regarded as an independent prognostic factor in several neoplasms (eg, in hematologic malignancies).¹³

The *bcl-2* gene codes for an inner mitochondrial membrane protein that inhibits apoptosis.¹⁴ In normal epithelium, *bcl-2* protein is expressed in the basal cells, and this expression reflects its obvious protective function to the dividing basal cells by elevating the thresh-

old to apoptosis in the sensitive mitotic phase.¹⁵ It counteracts the apoptosis-inducing effects of *p53*,¹⁶ thus balancing the cellular homeostasis. Elevated *bcl-2* protein expression has been found in various tumors, including nasopharyngeal carcinoma.¹⁷

In this study, we collected data on all patients younger than 40 years in whom tongue cancer was diagnosed in Finland during a 10-year period. The risk factors, clinical behavior, and at least 5-year follow-up data were analyzed. The expression of *p53* and *bcl-2* proteins was analyzed by using immunocytochemical staining methods. The mutational status of *p53* was studied by using the nonradioactive single-strand conformation polymorphism (SSCP) technique. We also compared biologic data with those derived from our previous studies on older patients.

RESULTS

The ratio of tongue cancer in young adults younger than 40 years to all patients with tongue cancer was counted in 10-year periods, beginning from 1953, when the systematic registration in the Finnish National Cancer Registry had been started, and ending in 1992. During that period, the incidence of tongue cancer was almost doubled in the

antibody (DAKO-bcl-2, Dakopatts, Glostrup, Denmark), diluted 1:100 at 4°C overnight. After rinsing in phosphate-buffered saline solution, the sections were incubated with biotinylated secondary antibody (Vectastain ABC kit) at room temperature for 30 minutes; after washing, these sections were treated with avidin-biotin complex (Vectastain ABC kit). The samples were then incubated in diaminobenzidine at room temperature for 5 minutes, washed in running water, and counterstained with hematoxylin. For p53 staining, a human breast cancer biopsy sample that was previously shown to be p53-positive was used as a positive control. The same sample was also used as a negative control by omitting the primary antibody. For bcl-2 staining, sections from tonsils were used as positive and negative controls. Additional negative controls were sections of tongue carcinoma that were stained by omitting the primary antibody.

The results of immunohistochemical staining were first classified according to the signal intensity (ie, no, slight, moderate, or strong) and the amount of positive cells in cancer tissue. The localization of signals (ie, basal, parabasal, whole epithelium, or scattered) of positive cells was also recorded. All these parameters were then summarized, and the samples were classified into 4 categories: no, weak, moderate, or strong expression.

POLYMERASE CHAIN REACTION (PCR) AND SSCP TECHNIQUE

One to several 5-μm-thick sections of paraffin-embedded blocks were cut with a total area of 1 cm². The DNA was extracted through xylene, graded alcohol, and proteinase K handling. Exons 5 through 9 of p53 were studied. The primers that were used are given in **Table 3**. The PCR was performed in a 50-μL reaction that contained the PCR buffer (10-mmol/L TRIS-buffered chloride, 1.5-mmol/L

magnesium chloride, 50-mmol/L potassium chloride, 0.1% Triton X-100) (Finnzymes OY, Espoo, Finland), 15 μL of the target DNA solution, 1.25-mmol/L deoxynucleotide triphosphate, 20 pmol of each primer, and 1.0 U of DNA polymerase (Finnzymes OY). The amplification was done in an automated DNA thermal cycler (Perkin-Elmer Corp, Norwalk, Conn) by using either 35 (exon 7) or 40 (exons 5, 6, and 8/9) cycles. The following program was used: the initial denaturation at 95°C for 4 minutes 30 seconds, followed by denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 1 (exon 7) and 2 (exons 5, 6, and 8/9) minutes. The final extension step was carried out at 72°C for 10 minutes. The successful amplification was confirmed by agarose gel electrophoresis (Nusieve, FMC Corp BioProducts, Rockland, Me) under UV light. Reamplification was done when no PCR product was visible on the gel. The reamplification was performed in the same way, except 1 μL of the PCR product was used. This method was applied on 2, 3, 6, and 9 samples of exons 5, 6, 7, and 8/9, respectively.

The SSCP technique was performed by mixing 2 μL of the PCR product with an equal volume of formamide-loading dye. The DNA was denatured by heating at 95°C for 5 minutes, followed by cooling on ice. Electrophoresis was carried out by using the PhastSystem (Pharmacia LKB, Uppsala, Sweden). The DNA fragments that contained exon 5 were run in a 12.5% gel at 15°C for 200 V hours, exon 6 in a 12.5% gel at 15°C for 250 V hours, exon 7 in a 20% gel at 15°C for 200 V hours, and exon 8/9 in a 12.5% gel at 20°C for 300 V hours. After electrophoresis, gels were silver-stained in its development unit.

For cross-tabulations, we used the χ^2 and Fisher exact tests. Survival times were analyzed by using the Cox model for proportional hazards. The computation was carried out by using commercially available statistical software (BMDP version 1993) on a mainframe (VAX/VMS).

whole population: from 302 cases between 1953 and 1962 and 585 cases between 1983 and 1992. This tendency was seen also in younger patients: there were 16 patients with tongue cancer younger than 40 years between 1953 and 1962 and 42 patients between 1983 and 1992. The percentages of younger patients were as follows: 1953 to 1962, 5.3%; 1963 to 1972, 4.3%; 1973 to 1982, 8.6%; and 1983 to 1992, 7.2%. The number of patients with new tongue cancers who were younger than 40 years fluctuated from 1 to 6 annually during the period from 1980 to 1989, which was the focus of this study.

The mean age of the patients was 32.3 years (age range, 19-39 years). The male-female ratio was 2:1. One patient had Fanconi anemia, and another had diabetes; all other patients were healthy. Before the diagnosis of cancer was made, 6 patients had been followed up because of leukoplakia or lichenoid changes of the oral mucosa. In 31 patients, the squamous cell cancer was located in the mobile part of the tongue; in 3 others, it was located at the base of the tongue. Only 8 patients (23.5%) presented with more advanced (T3-T4) disease, and, in 24 biopsy specimens (70.6%), carcinoma was classified as well differentiated. At the time of diagnosis, 13 patients (38%) had neck metastases. The overall 5-year survival of the patients was 71% (24/34). Of those patients (n=23) with a follow-up time that

ranged from 5 to 15 years, only 3 additional patients died: 2 died of the recurrence of the primary tumor and 1 died of the second primary tumor in the esophagus. The clinical course of the patients who were followed up for premalignant lesions of the oral mucosa was the same as that of other patients: 2 of 6 died during the total follow-up period.

One paraffin-embedded block that was obtained from 1 patient was missing; subsequently, the p53 and bcl-2 analyses were carried out for 33 patients. Immunopositivity for p53 was strong in 5 (15%), moderate in 6 (18%), weak in 13 (39%), and absent in 9 (27%) of the cancer biopsy specimens. The p53 staining was always intranuclear and often present also in the basal cells of the normal epithelium. In malignant epithelium, the p53 expression was often found in the entire epithelium, especially in the sections with strong positivity (**Figure 1**).

Strong immunopositivity for bcl-2 protein was not found in any of the biopsy specimens, but it was moderate in 10 (30%), weak in 7 (21%), and absent in 16 (48%) of the cases. The staining was cytoplasmic and also present in most of the mitotic cells (both normal and malignant). The expression was found in the basal cell layer of the normal epithelium, but it mostly disappeared when the epithelium became dysplastic or malignant (**Figure 2**). Lymphocytes showed intensive staining.

Table 1. Pertinent Clinical Data, Immunohistochemical Staining, and *p53* Mutations in Carcinomas of the Tongue*

Patient No./Sex/Age, y	TN Stage	Cancer Grade	Clinical Outcome	Survival/Follow-up†	Type		Staining Pattern		Mutations of <i>p53</i>
					Smoking	Alcohol	<i>p53</i>	<i>bcl-2</i>	
1/M/36	T2, N1	1	DOD	18 mo	MS	MC	0	0	Exon 6
2/M/38	T1, NO	2	DOSP	96 mo	HS	MC	2	1	Exon 8/9
3/F/32	T1, NO	1	NED	5 y	NS	MC	1	0	Exons 6 and 7
4/M/39	T3, NO	1	NED	5 y	HS	MC	3	0	Exon 5
5/M/33	T2, N1S	3	DOD	87 mo	HS	MC	1	2	Exon 7
6/M/37	T2, NO	2	DOD	12 mo	1	0	Exon 8/9
7/F/31	T2, NO	1	NED	10 y	1	0	Exons 6 and 7
8/M/33	T4, N1	1	DOD	13 mo	NS	MC	2	0	None
9/M/34	T1, NO	1	NED	6 y	HS	...	1	2	None
10/F/34	T2, N1	1	NED	7 y	0	2	None
11/M/39	T4, N1S	1	DOD	8 mo	HS	HC	2	0	Exon 5
12/F/39	T1, NO	2	NED	8 y	NS	MC	BNA	BNA	BNA
13/F/30	T3, NO	1	NED	6 y	MS	...	3	2	None
14/F/38	T1, NO	1	NED	7 y	HS	MC	3	2	Exon 7
15/F/32	T2, N1	1	NED	9 y	MS	MC	0	2	None
16/F/19	T2, NO	1	DOD	22 mo	1	1	None
17/M/34	T1, NO	2	DOA	2 mo	HS	...	0	0	None
18/F/34	T1, NO	1	DOD	142 mo	NS	MC	2	2	Exon 7
19/M/37	T2, N1	1	NED	7 y	HS	MC	0	1	Exon 5
20/F/27	T1, NO	2	NED	7 y	1	1	None
21/M/19	T1, NO	1	NED	6 y	1	1	None
22/F/25	T2, NO	1	NED	11 y	HS	...	1	2	Exon 6
23/M/26	T3, NO	1	NED	6 y	MS	HC	0	1	Exon 5
24/M/24	T1, NO	2	DOD	30 mo	0	2	None
25/M/28	T4, N3S	2	DOD	1 mo	3	2	Exon 7
26/M/22	T1, NO	1	NED	7 y	1	1	Exon 6
27/M/25	T2, NO	1	NED	10 y	HS	...	2	0	None
28/M/37	T1, NO	1	NED	11 y	0	0	None
29/M/38	T3, N1	1	NED	7 y	HS	...	3	0	Exon 8/9
30/M/39	T2, N1	1	DOD	7 mo	NS	MC	0	0	None
31/F/36	T1, NO	2	NED	9 y	NS	MC	1	0	Exon 8/9
32/M/32	T3, N1	1	DOD	10 mo	NS	MC	1	0	None
33/M/38	T2, N1	1	NED	7 y	NS	MC	1	0	None
34/M/34	T1, N1	3	DOD	7 mo	NS	MC	2	0	None

* T indicates tumor; N, node; DOD, dead of disease; MS, moderate smoker; MC, moderate consumer; DOSP, dead of second primary tumor; HS, heavy smoker; NED, no evidence of disease; NS, nonsmoker; HC, heavy consumer; BNA, block not available; and DOA, dead of accident.

† Survival is given in months; follow-up is given in years.

‡ Immunohistochemical staining patterns were scored as follows: 0, no staining; 1, weak staining; 2, moderate staining; and 3, strong staining.

\$ Base of tongue cancer.

Table 2. TN Classification and Histologic Grade of Tumors*

T Stage	N Stage		Grade			Total
	NO	N1-N3	1	2	3	
T1	13	1	7	6	1	14
T2	5	7	10	1	1	12
T3	3	2	5	0	0	5
T4	0	3	2	1	0	3
Total	21	13	24	8	2	34

* T indicates tumor; N, node.

A statistically significant association was found between the expression of *p53* and tumor size ($P < .05$): of 8 tumors (T3-T4), 6 (75%) showed moderate to strong staining, in contrast to 5 (20%) of 25 tumors (T1-T2). The *p53* expression was not associated with the histologic grade of carcinoma, neck metastases, smoking, or

Table 3. Primers Used for PCR*

Exon	Sequence	Size, bp
5A	5'-TTC CTC TTC CTG CAG TAC TC-3'	214
5B	5'-GCC CCA GGT GCT CAC CAT CG-3'	
5'6A	5'-ACC ATG AGC GCT CCT CAG AT-3'	236
6B	5'-AGT TGC AAA CCA GAC CTC AG-3'	
7A	5'-GTC TTG TCT CCT AGG TTG GC-3'	138
7B	5'-CAA GTG GCT CCT GAC CTG GA-3'	
8A	5'-GCT ATC CTG AGT AGT GGT AA-3'	331
9B	5'-CCC AAG ACT TAG TAC CTG AA-3'	

* PCR indicates polymerase chain reaction; bp, base pair.

alcohol consumption. The *bcl-2* staining did not correlate to any of these parameters.

The SSCP analysis of *p53* mutations was performed in all 33 available samples. The SSCP analysis of exons 5 to 9 of *p53* was successful in 17 patients. Exon 8/9 could not be amplified in 16 samples, while exons 5, 6, or 7

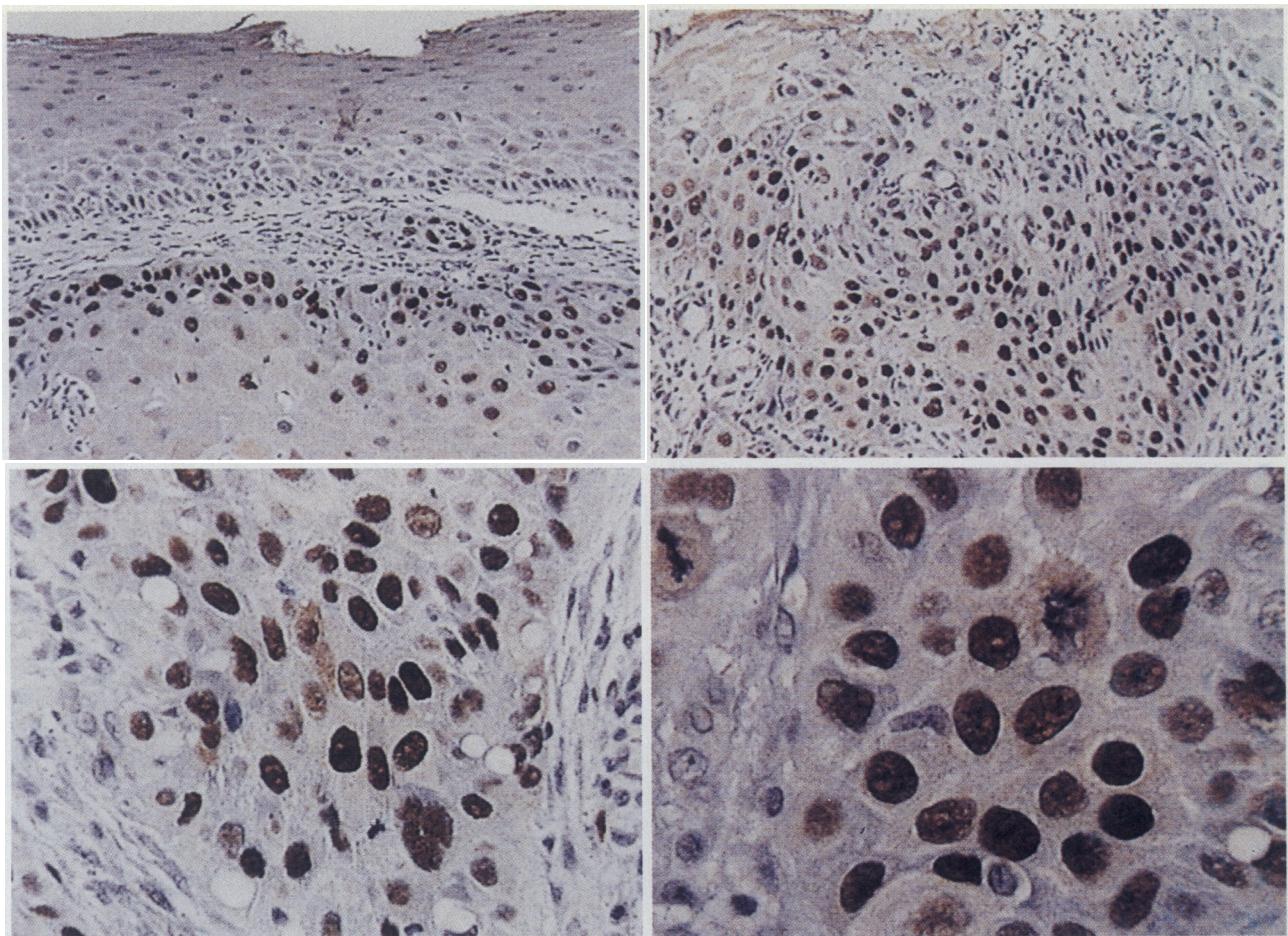


Figure 1. A low-power micrograph of p53 immunostaining depicting nuclear expression in normal epithelium and squamous cell carcinoma (original magnification, $\times 40$ [top left]). Intense nuclear staining is shown in squamous cell carcinomas (original magnification, $\times 100$ [top right], $\times 250$ [bottom left], and $\times 400$ [bottom right]). No signals are present in the normal overlying epithelium, whereas the cancer tissue shows strong signals. (Staining with diaminobenzidine, counterstaining with hematoxylin.)

failed to amplify in 7 samples. It is likely that the length of exon 8/9, combined with the small size of the samples or, alternatively, inhibitory factors in paraffin-embedded blocks, were responsible for this failure. An altered electrophoretic pattern suggestive for the p53 mutation was found in 17 patients (52%) (Table 1) (**Figure 3**). The mutations were evenly distributed in different exons: 4 in both exon 5 and exon pair 8/9, 5 in exon 6, and 6 in exon 7. In 2 cases, 2 different mutations were found in the same sample. All 4 mutations in exon 5 were found among the smokers and heavy alcohol consumers. Two patients who died of primary tongue cancer after the 5-year follow-up had a p53 mutation detectable in the SSCP. The sample that was taken from the patient who was dying of the second primary cancer in the esophagus carried also a p53 mutation. The p53 expression was not associated with the p53 mutations. Similarly, no correlation of p53 mutations with the tumor size, grade, neck metastases, smoking, or alcohol consumption could be detected.

The survival was significantly associated with the grade ($P=.01$) and size of the tumor ($P=.01$). There was a tendency ($P=.09$) for an interaction between the grade and p53 mutations toward shortening of the survival.

COMMENT

Based on the files of the Finnish National Cancer Registry, this series included all patients younger than 40 years in Finland who were diagnosed as having a tongue cancer during a 10-year period (1980-1989). In addition, the longtime statistics, covering 40 years from 1953 to 1992, of tongue cancer incidence and the percentage of younger patients were collected. During this study period, the total incidence of tongue cancer almost doubled, and this was also reflected in the incidence of younger patients. There was a slight increase in the ratio of tongue cancer in younger patients from 5.3% (in the 1950s) to 7.2% (in the 1980s) that was presumably not significant because the ratio was even higher for those patients in the 1970s, namely, 8.6%. The overall increase in the number of cases of tongue cancer also may have been owing to the more accurate registration in the 1970s and 1980s.

It has been estimated that smoking and alcohol consumption accounted for 75% of all cases of oral squamous cell cancers. However, the significance of these risk factors among young patients is still controversial. In this study, 58% of the patients with available smoking data

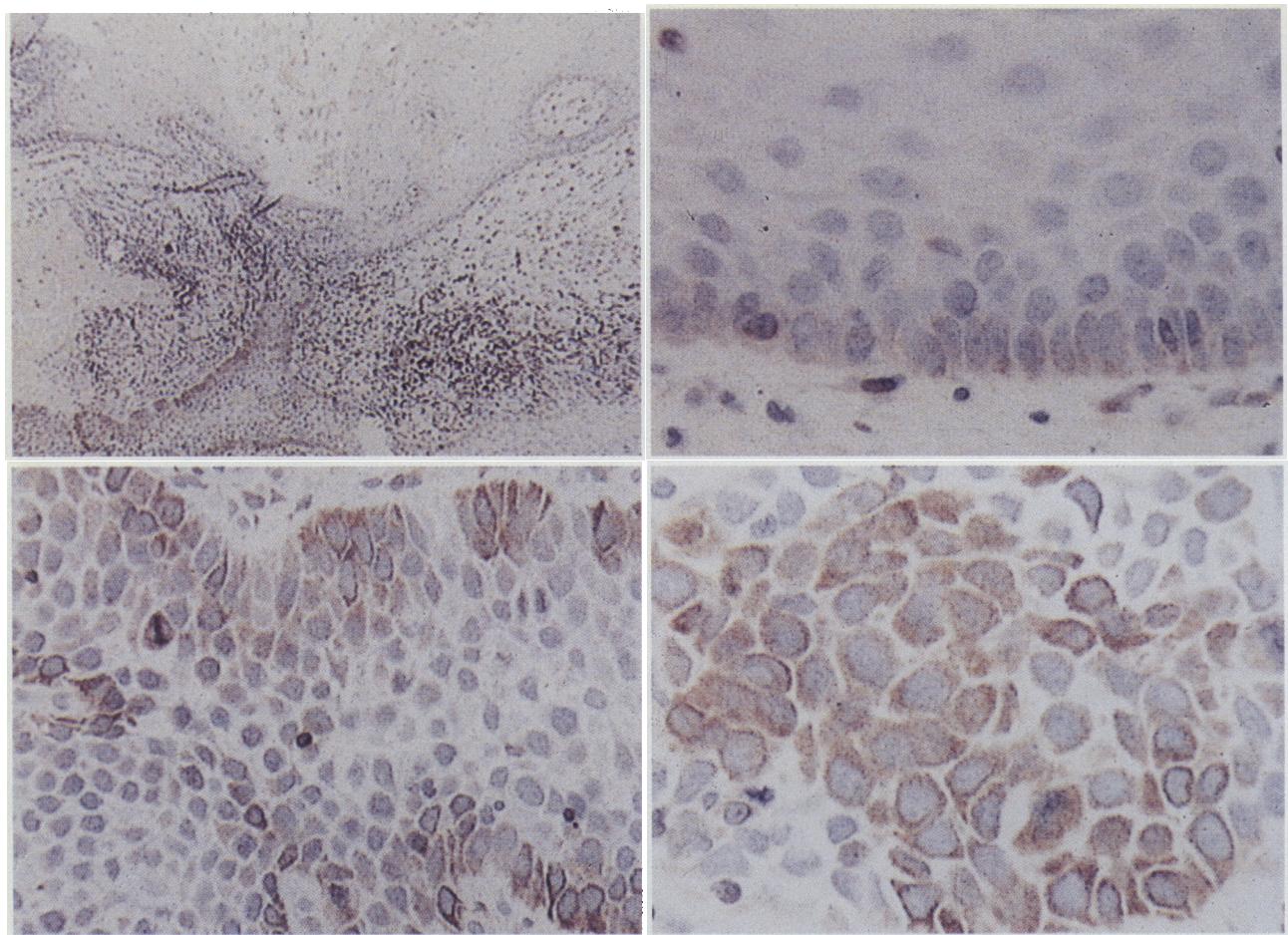


Figure 2. Results of bcl-2 immunohistochemical staining techniques. Top left, A normal epithelium with malignant transition. The most intense signals are found in the cancer tissue (original magnification, $\times 25$). Top right, Cytoplasmic staining found in the basal cells of normal epithelium (original magnification, $\times 250$). Carcinoma cells are strongly stained (original magnification, $\times 250$ [bottom left], $\times 400$ [bottom right]). (Staining with diaminobenzidine, counterstaining with hematoxylin.)

were moderate or heavy smokers, but only 2 were heavy drinkers. Because of their young age, the carcinogens of tobacco might not have had enough time to give rise to a malignant change in many of the patients. However, it can be speculated that exposure of the epithelium to carcinogens at a young age may reduce the period of latency of carcinogenesis.¹⁸ Nevertheless, patients without any known risk factors must have had other causes for the disease. Our study suggests that genetic or immunologic disorders might be of importance. One patient had Fanconi anemia, and another, who died of cancer at the age of 21 years, had a brother who had died of Fanconi anemia. Interestingly, a sister of 1 of the patients also had tongue cancer; this finding further suggests the possible role of familial genetic defects in oral carcinogenesis.

Recently, it was found that p53 mutations in head and neck tumors were more frequent in patients in their 60s than in patients older than 75 years.¹⁹ Also, these older patients usually had a history of less extensive cigarette smoking and less alcohol consumption. Accumulation of spontaneous mutations during the lifetime and defects of the DNA repair machinery may play more important roles in the carcinogenesis in very old people; these

factors support the concept of separate progenitors underlying the oral cancer in the entire life span of people.

The proportion (70.6%) of well-differentiated carcinomas was surprisingly high in our series. The reason for this is unknown, but it may explain in part the reported favorable outcome among our patients. At the time of diagnosis, the TNM staging,²⁰ as well as the frequency of neck metastases, resembled that found in the oral cancer of older age groups. The 5-year survival for diseases (T1-T2) was 73.1%; this finding is in accordance with that found in patients with tongue cancer in general.²¹ For T3 and T4 tumors, the 5-year prognosis was 50%, with this being slightly better than that reported in patients with tongue cancer of a normal age variation.²² Interestingly, the clinical course of the disease in young patients was not worse than that found in older age groups.

A strong or moderate immunostaining for p53 was found in 33% of the patients; this finding was consistent with that of previous reports on tongue cancer.²³ The antibody that was used (CM1) recognized both the mutant and wild type of p53. The increasing intensity of p53 expression with the tumor size may have many explanations. It may reflect the more rapid growth of cancer. Alternatively, stabilized, functionally inactivated p53 may

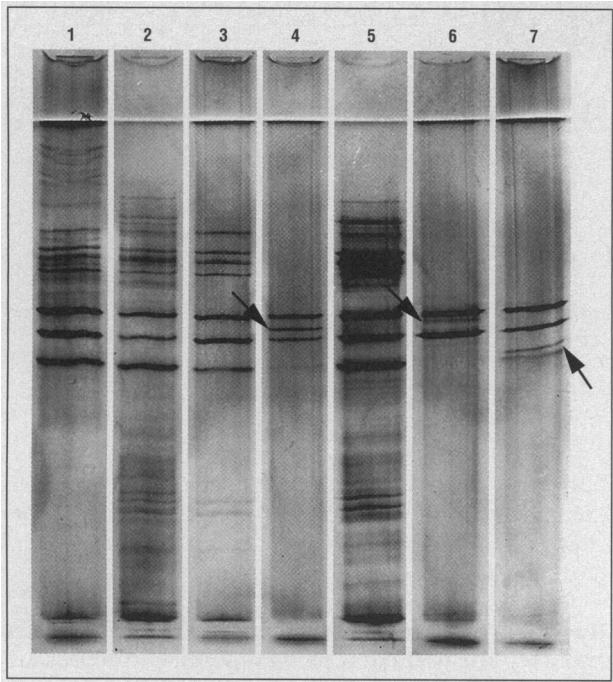


Figure 3. Gel electrophoresis screening of the mutations in exon 7 of p53 with the single-strand conformation polymorphism technique. An altered electrophoretic pattern (arrows) is evident in lanes 4, 6, and 7 (samples 5, 7, and 14, respectively). Lane 1 depicts control sample (DNA from human cervical carcinoma-derived CaSki cells).

accumulate into the cells of advanced carcinomas. Moreover, the attempts of the wild-type p53 to direct the cells to apoptosis may contribute to its higher expression at later stages. The samples that were taken from the patient with Fanconi anemia, who died of tongue cancer within 1 month, showed intense p53 and moderate bcl-2 staining, and the single exon 7 of p53 that could be amplified was mutated. These findings suggest an overall derangement of diverse molecular pathways in an advanced cancer.

Although the SSCP is a screening method, all mutations that were suspected by it so far have also been able to be confirmed by the direct sequencing in our group. The rate of p53 mutations in our study was similar to that reported by others.²⁴ However, we could not find any "hot-spot" exon as reported in the previous studies. Interestingly, all mutations in exon 5 were found in cigarette smokers; that group also included the heavy drinkers. The association between exon 5 mutations and non-small-cell lung cancer, which is a tobacco-related disease, has been suggested recently.²⁵ Similarly, in our unpublished series, we found that exon 5 seemed to be a mutational hot spot in tongue cancer in elderly patients. Exon 5 of p53 gene might be the site for the exogenous risk factors to leave their chromosomal "fingerprints."

The most adverse prognosis, accumulating in the patients with both p53 mutation and moderately or poorly differentiated squamous cell cancer, implicates the role of p53 as a potential molecular prognostic marker in tongue cancer that is capable of distinguishing the high-risk patients. It is to be emphasized that the present results are based on a limited number of patients and should be interpreted with caution. However, it seems that p53 might function as an adjunct prognostic factor and pro-

vide the clinician with a genetic tool that can assist him or her in the selection of individual therapy.

Accepted for publication July 22, 1996.

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