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COMPARATIVE GENOMIC HYBRIDIZATION ANALYSIS OF TONSILLAR CANCER REVEALS A DIFFERENT PATTERN OF GENOMIC IMBALANCES IN HUMAN PAPILLOMAVIRUS-POSITIVE AND -NEGATIVE TUMORS

Liselotte DAHLGREN[1]*, Hanna MELLIN[1], Danny WANGSA[2], Kerstin HESELMEYER-HADDAD[2], Linda BJÖRNESTÅL[3], Johan LINDHOLM[1], Eva MUNCK-WIKLAND[3], Gert AUER[1], Thomas RIED[2] and Tina DALIANIS[1]

[1]*Department of Oncology-Pathology, Karolinska Institute, Karolinska Hospital, Stockholm, Sweden*
[2]*Genetics Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA*
[3]*Department of Otorhinolaryngology, Head and Neck Surgery, Karolinska Institute, Karolinska Hospital, Stockholm, Sweden*

Our aim was to map and compare genomic imbalances in human papillomavirus (HPV)-positive and -negative squamous cell carcinomas of the tonsil. Twenty-five primary carcinomas were analyzed by comparative genomic hybridization. Fifteen (60%) were found to be HPV-positive by PCR, and the majority were HPV-16. There were statistically significant differences in the distribution of DNA gains and losses between the HPV-positive and -negative samples. Eleven of 15 HPV-positive samples (73%) showed gain on chromosome 3q24-qter, while only 4/10 (40%) HPV-negative samples had the same gain ($p = 0.049$). Furthermore, 4/10 (40%) HPV-negative samples but no HPV-positive samples had gain on chromosome 7q11.2-q22 ($p = 0.017$). As expected, and similar to previous studies, patients with an HPV-positive tumor had a statistically significantly better disease-specific survival than patients with an HPV-negative tumor ($p = 0.002$). The most common changes, *e.g.*, gain on 3q or 8q, loss on 11q or 13 and loss on chromosome 7q in HPV-negative tumors, did not have any influence on prognosis. However the number of cases in each subgroup was limited.

Key words: *human papillomavirus; tonsillar cancer; comparative genomic hybridization; chromosome 3q*

Head-and-neck cancer constitutes 3.4% of all cancer cases each year in Europe[1] and is the fifth most common cancer type in the United States.[2] In approximately 50–60% of patients, the tumor has spread to regional lymph nodes by the time of diagnosis, and it is known that formation of metastases reduces the chance of survival by about 50%.[2] Treatment of head-and-neck cancer has not improved greatly over the last years, and the 5-year survival rate remains low.[3] The main reasons for the low survival rate are advanced tumor stage at detection, high prevalence of recurrence and multiple primary tumors.[3]

The major risk factors of head-and-neck squamous cell carcinoma (HNSCC) in the Western world are smoking and alcohol consumption. However, during the past 2 decades the role of high-risk human papillomavirus (HPV) has been studied, and data supporting HPV as a causative agent in the development and progression of a subset of these cancers have accumulated.[4–6] The overall frequency of HPV in HNSCC is around 25–30%, with considerable variability depending on the tumor location.[7] The highest frequency is reported from studies on tonsillar cancer,[7–9] where 35–70% of tumors are HPV-positive, most commonly with HPV-16 and/or HPV-33. Furthermore, we and others have shown that patients with HPV-positive tonsillar cancer have a statistically significant reduction in risk of death from cancer compared to patients with HPV-negative tumors and that this is independent of tumor stage.[4,8] In addition, in a relatively limited study, patients with high viral load tumors had a significantly better prognosis compared to patients with low viral load tumors.[10]

The fact that HPV is a favorable predictive/prognostic factor in tonsillar cancer prompted us to analyze whether differences in the pattern of chromosomal gains and losses were correlated with the presence of HPV. We hypothesized that such differences could explain the variable clinical course of HPV-positive cancer.

In high-risk HPV-positive cancer of the tonsil, the early proteins E6 and E7 are generally expressed,[11] and it is known that the presence of these proteins is sufficient to immortalize and transform keratinocytes.[12] In cervical cancer, constant expression of E6 and E7 is required for malignancy.[13] E6 and E7 interfere with p53 and pRb, respectively, leading to cell-cycle progression and accumulation of genetic damage.[13–15] E6 binds and initiates degradation of p53 and activates *c-myc*, leading to activated telomerases,[16] while the E7 protein forms complexes with proteins in the *Rb* gene family, liberating the E2F transcription factor and promoting cell division.[17]

Furthermore, it is known that multiple genetic changes are involved in the development of HNSCC and that the karyotypes observed in HNSCC are among the most complex so far described in solid tumors.[18] Losses involving 9p, 3p and 17q have been identified in preneoplastic lesions; and 9p and 3p are also the most frequently reported losses in established HNSCC.[19] The target genes for some chromosomal losses are known. For instance, the *p16* gene maps to chromosome band 9p21, and *p53* localizes to 17p13.[20] Also, inactivation of *p16*, by mutation, loss of heterozygosity or hypermethylation, is an early event, which occurs in 59% of HNSCC cases.[21] Gain of 3q is an early sign of metastatic disease, and gain of 1q and 2q usually results in a clinically poor outcome.[22] Other examples of genetic changes are somatic mutations of *p53* (17p13), which are detected in 25–50% of HNSCCs, notably in HPV-negative tumors, and expression of *cyclin D1*, which has been described to be inversely proportional to HPV infection.[23] In addition, DNA gains that map to 3q26-qter, 5p14-pter and 8q are frequent in HNSCC.[24,25]

Although several studies have been performed regarding the genetic imbalances in HNSCC,[18,25] to our knowledge none has correlated genomic imbalances with the presence or absence of HPV.

We compared genetic imbalances in 25 fresh-frozen HPV-positive and -negative tonsillar carcinomas. HPV status was investigated by PCR using the consensus primers GP5+/6+[26] and CPI/CPIIG.[27] Sequencing and/or type-specific HPV PCR was

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*Correspondence to: Department of Oncology-Pathology, Cancer Center Karolinska, R8:01, Karolinska Institute, Karolinska Hospital, SE-171 76, Stockholm, Sweden. Fax: +46-8-309195.
E-mail: Liselotte.Dahlgren@cck.ki.se

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performed for HPV typing. Chromosomal imbalances were identified by comparative genomic hybridization (CGH).[28]

MATERIAL AND METHODS

Patient samples and clinical background

Forty fresh-frozen, pretreatment biopsies were obtained at the time of diagnosis from patients with primary squamous cell carcinomas of the tonsil, diagnosed 1993–2001 at the Department of Otorhinolaryngology, Head and Neck Surgery, Karolinska Hospital. All patients gave informed consent, and ethical permission according to the Karolinska Institute was obtained. A pathologist confirmed the diagnosis on hematoxylin/eosin sections and calculated the percentage of cancer cells in the material. TNM stage classification was done according to the UICC system, and the differentiation grade was according to the WHO international histologic classification of tumors. Clinical data for the 25 patients included in the study were obtained from the files of Radiumhemmet and the Department of Otorhinolaryngology, Head and Neck Surgery, Karolinska Hospital. For a summary of the patient data and the tumors, see Table I.

Extraction of DNA

DNA was extracted from 2 × 50 μm frozen tumor sections. Before, between and after the 2 sections, a 5-μm-thick section was taken for staining with hematoxylin/eosin for tumor tissue verification. To avoid HPV contamination, an empty block was cut and sections were collected between every tumor block. DNA was extracted according to either the high-salt extraction protocol or a standard phenol extraction protocol (for protocol details, see www.riedlab.nci.gov/protocols). In some cases, DNA was extracted from paraffin-embedded biopsies.

TABLE I – CHARACTERISTICS AND CLINICAL DATA OF THE PATIENTS

Characteristic	HPV-positive	HPV-negative
Number	15	10
Age at diagnosis (mean, years)	29–84 (62.5)	42–87 (66.8)
Sex		
Male	12	7
Female	3	3
Smoking habit		
Never smoked	6	1
Ex-smoker	5	0
Smoker	3	7
Not known	1	2
Alcohol consumption		
Normal	7	1
Heavy	1	5
Not known	7	4
TNM stage[1]		
I	0	1
II	0	0
III	3	1
IV	12	8
Histopathologic grade[2]		
Well differentiated	0	0
Moderately differentiated	8	5
Poorly differentiated	6	4
Moderately/poorly	0	1
Unknown	1	0
Primary treatment		
Preoperative radiotherapy	13	5
Postoperative radiotherapy	0	0
Radiotherapy only	1	2
Surgery only	0	1
No or palliative treatment only	1	2
Response to radiotherapy		
Complete	7	4
Partial	7	2
None	0	1

[1]TNM stage, tumor stage according to UICC.–[2]Tumor differentiation grade according to WHO international histologic classification of tumors.

Verification of amplifiable DNA by S14 PCR

All tumor samples were run in S14 PCR for verification of amplifiable DNA. The PCR mixture consisted of 5 μl 10× PCR buffer (Applied Biosystems, Foster City, CA), 200 μM of each dNTP (Applied Biosystems), 1.5 mM $MgCl_2$, 4 μg/μl BSA, 15 pmol of each S14 primer (kindly provided by Dr. K.-L. Wallin, Department of Molecular Medicine, Karolinska Institute, Karolinska Hospital, Stockholm, Sweden), 10 U Taq polymerase (Applied Biosystems) and 100–200 ng sample DNA in a final volume of 50 μl. Water was used as the negative control, and DNA extracted from normal tonsillar tissue was used as the positive control. Amplification was run in an automated thermocycler (Perkin-Elmer, Norwalk, CT) and initiated by a denaturation step at 94°C for 1 min, followed by 40 amplification cycles consisting of denaturation at 94°C for 30 sec, annealing at 50°C for 30 sec and elongation at 72°C for 45 sec. In the final cycle, the elongation step was extended to 5 min. PCR products were run on a 2.5% agarose gel stained with ethidium bromide and visualized with UV light.

HPV detection by PCR

A general HPV PCR was run with 100–200 ng sample DNA, as previously described, with general primers GP5+/6+. The excess of cloned plasmids of either HPV-6 (100,000 copies) or -16 (1,000 copies) was used as the positive control, and water was used as the negative control. Products were detected as above, and samples with a band at 130–150 bp were considered positive and run in an HPV type-specific PCR. For verification of the GP5+/6+ PCR protocol sensitivity, fresh-frozen SiHa (known to harbor 1–2 copies of integrated HPV-16 per genome) was diluted in water in 10-fold steps and run as above. Positive results were obtained in samples with a minimum of 1.5 ng SiHa DNA.

Samples negative in the GP5+/6+ PCR were run in another general PCR with the CPI/CPIIG primer pair,[27] to exclude false-negatives as a result of disrupted L1. The PCR was run as previously described.[10] Products were detected as above, and samples with a band at 187 bp were considered HPV-positive and run in an HPV type-specific PCR. Verification of the CPI/IIG PCR protocol sensitivity was run as above, and positive results were obtained in samples with a minimum of 15 ng SiHa DNA.

HPV type-specific PCR

Samples considered HPV-positive were run in an HPV type-specific PCR with primers specific for HPV-16 and (if not HPV-16-positive) for HPV-18 and HPV-33,[29] as previously described.[10] Positive controls consisted of an excess of plasmids with cloned HPV-16, -18 or -33. Products were detected as above, and samples with a band at 120 bp for HPV-16, 172 bp for HPV-18 and 211 bp for HPV-33 were considered positive. Verification of the HPV type-specific PCR protocol sensitivity was run as above, and positive results were obtained in samples with a minimum of 0.15 ng SiHa DNA. Some positive samples were confirmed by sequencing as previously described.[10]

CGH

CGH was carried out as previously described.[30] In short, tumor and control DNA were labeled by nick translation with biotin-dUTP and digoxygenin-11-dUTP (DIG), respectively; and 500 ng–1 μg nick-translated test and control DNA were coprecipitated in 3 M NaAc and ethanol using an excess of human Cot-1 DNA (Invitrogen, La Jolla, CA) and salmon sperm DNA (Sigma, St. Louis, MO). The precipitate was resuspended in 5 μl deionized formamide (pH 7.5) at 37°C for 1 hr, and then an equal volume of master mix (20% dextran sulfate and 2 × SSC, pH 7.0) was added to the probes, followed by incubation at 37°C for 30 min. Probes were denatured at 85°C for 5 min, followed by preannealing at 37°C for 1–2 hr. Normal lymphocytes from healthy donors were stimulated with phytohemagglutinin and used for metaphase spreads. Probes were hybridized to metaphase slides for 72 hr at

37°C and detected with avidin-conjugated FITC antibodies for test DNA and anti-DIG antibodies conjugated to tetramethylrhodamine isothiocyanate (TRITC) for control DNA. Chromosomes were stained with 4,6-diamidino-2-phenylindole. At least 10 metaphases per case were imaged using a Leica (Cambridge, UK) DM RXA microscope with a cooled CCD camera (Sensys; Roper Scientific, Tucson, AZ). The ratio between the FITC and TRITC intensities was analyzed with CW 4000 software from Leica. FITC:TRITC ratios >1.2 defined a gain and those >1.4, an amplification. Ratios <0.8 defined loss of genomic material.

Statistical analysis

Fisher's exact 2-tailed test was used to correlate the frequency of HPV and chromosomal imbalances to clinical data. Logistic regression was used to estimate if the correlation of HPV and genetic imbalances with clinical data influenced the outcomes of the patients. Survival analysis was done using the Kaplan-Meier method. Significance between differences in survival rate was analyzed by the log-rank test. Cox regression (uni- and multivariate) was used to evaluate factors influencing mortality risk. All analyses were performed using Statistica software (StatSoft, Tulsa, OK).

RESULTS

Screening of HPV frequency and typing of HPV in tonsillar cancer

Twenty-five samples were screened for the presence of HPV DNA. Of these, 15 (60%) were HPV-positive, as determined using either the L1-specific consensus primers GP5+/6+ (13 samples) or the E1-specific consensus CPI/CPIIG primer pair (2 samples). The distribution of HPV is described for the whole group in Table I and for individuals in Table II. HPV type-specific PCR and sequencing showed that 14 samples harbored the high-risk HPV type 16. One sample was not typed due to lack of material.

CGH

Twenty-five of the 40 biopsies gave reliable profiles when analyzed by CGH. Only samples that contained at least 70% tumor cells were used. Four tumors showed no genomic imbalances, while the remaining 21 samples displayed up to 14 regions showing gains and up to 9 regions with losses, excluding changes on the sex chromosomes. The results of CGH analysis of all cases are summarized in Figure 1. Mean numbers of gains and losses per case were 3.0 and 2.1, respectively, giving an average number of chromosomal aberrations (ANCA)[31] of 5.2. Changes could be detected on all chromosomes except for chromosome 6. The most common gains were observed on chromosome arms 3q in 60%, 8q in 32%, 20q in 24% and 11q, 12p and 17q in 20%, while the most common losses were seen on chromosomes 11q14-qter (in 44%) and 13 (in 20%). The specific gains and losses for each tumor are described in Table II.

Among the 15 HPV-positive tonsillar cancers (Fig. 2, pink bars), the gains ranged between 0 and 10 per case, with a mean at 2.3, and the losses between 0 and 6 per case, with a mean at 2.1, which results in an ANCA value of 4.5. The most frequently occurring gain in this tumor group was seen on chromosome 3q23-qter, where 11/15 cases (73%) had an increased copy number (Figs. 2, 3*a*), whereas the most frequently occurring loss (7 cases, 47%) was seen on chromosome 11q14-q25 (Figs. 2, 3*b*). Amplifications were seen for 3 cases on chromosome 3q with the minimal region 3q24-q27 and on chromosomes 8, 9 and 20p in one tumor each (Fig. 2).

Among the 10 HPV-negative samples (Fig. 2, blue bars), the gains ranged between 0 and 16, with a mean at 4.0, and the losses ranged between 0 and 9, with a mean at 2.1, resulting in an ANCA value of 6.1. The most frequent gains were seen on chromosomes 3q26.1-qter, 7q11.1-22 and 8q24.3, where 4 cases (40%) showed copy number increases (Figs. 2, 3*a*). Two amplifications were seen in this subgroup, one on chromosome 7q and one on chromosome 17q11.2-q12 (Fig. 2). The most frequent loss mapped to chromosome 11q14-q25, where 4 cases (40%) had copy number losses (Figs. 2, 3*b*). All profiles have been submitted to the NCBI SKY/CGH database (www.ncbi.nlm.nih.gov/sky/).

TABLE II – HPV AND CGH DATA OF 25 SQUAMOUS CELL CARCINOMAS OF THE TONSIL

Case[1]	Response[2]	HPV	DNA losses	DNA gains
2-2548	CR	+	—	3q13.1-q29
3-2549	CR	+	—	—
4-2551	CR	+	3pter-p12, 9pter-p12, 11q13-q25, 18pter-p11.2, 18q21-q23	3q23-q29, 5pter-p12, 11q12-q13, 12pter-p11.2, 18q11.1-q21
5-2552	PR	+	—	11q12-q13
7-2553	PR	−	3p, 4p, 5, 9p, 10p, 11q13-q25, 13, 15, 18q	3q, 7q, 8q24.3, 11q12-q13, 12pter-p11.2, 14, 19q, 20, 22q13-q13
9-2759	PR	+	13, 16q	3q21-qter, 8q23-qter
10-2557	CR	+	2q35-q37, 3p22-p12, 11q, 13, 14q24-q32, 16	3q, ++3q24-q27[3] 5, 8, ++9p, ++9q13-q34, 10, 11p12, 12, 17, 18
11-2758	PR	−	11q14-qter, 13, 21	1q21-q41, 3q26.1-qter, 8q21.1-qter, 11q13, 18q, 20q
13-2559	PR	−	—	—
14-2560	PR	+	11q13-q25	3q
15-2561	CR	+	4p, 11q13-q25, 16q	16p, 17
17-2562	CR	−	—	7q11.2-q31
18-2564	PR	+	4q28-q35, 13	—
22-2568	CR	+	7p, 7q11.1-q21, 14q, 16q	3q, 8, 10, ++20p, 20q
23-2569	CR	−	—	—
25-2578	NR	−	7q22-q36, 10q23-q26	3q, 7p21-p11.2, 7q11.1-q22
28-2571	NR	+	3p, 14	3q, 20p
30-2572	NR	+	11p, 11q14-q25, 18q21-q23	++3q, ++8, 10p, 20
32-2579	—	−	—	—
36-2573	CR	−	10q, 11q13-q25	3q, 17, 20q, 22
38-2760	—	−	4, 11q13-q25, 13, 18q12-q23	1pter-p34.3, 1q, 2q11.1-q31, ++7q, 8q22-q24.3, 9p24-p12, 11q11-q13, 12p, 14q22-q32, 15, 16p, 17q, ++17q11.2-q12, 18q11.1-12, 19p
40-2581	CR	−	2q14.3-q23	8, 12, 17q25
41-2574	CR	+	10q, 11q	++3q
44-2576	PR	+	—	3
46-2577	—	+	11q13-q25, 20pter-p11.1	3q21-q29, 20q11.1-q13.3

[1]Case number and NCBI SKY/CGH database accession number.–[2]CR, complete response; PR, partial response; NR, no response.–[3]Amplified.

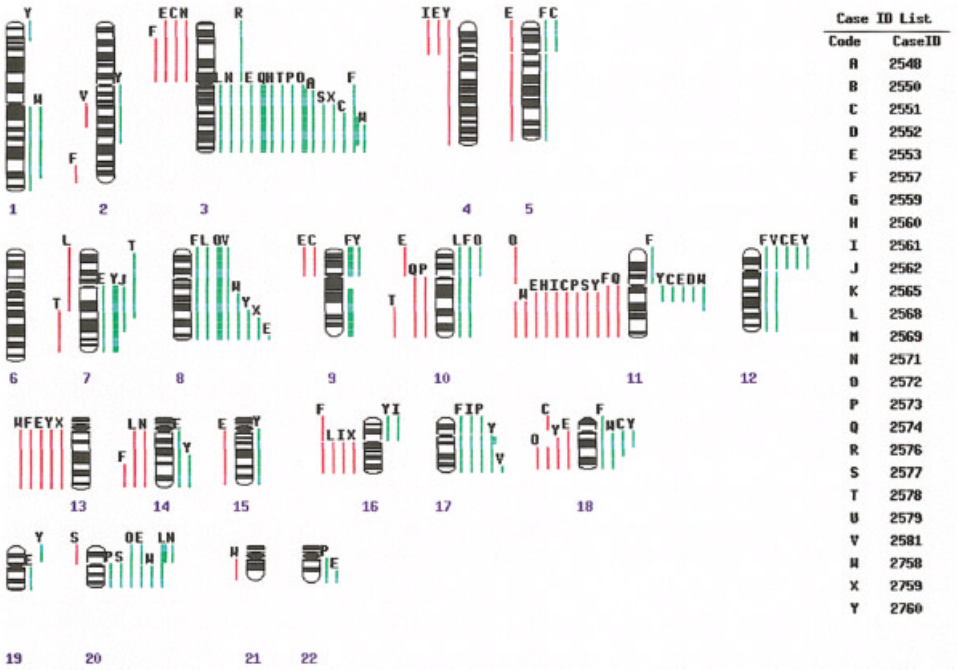


FIGURE 1 – Gains and losses in 25 squamous cell carcinomas of the tonsil. Green bars to the right of the chromosomes indicate gains; red bars to the left indicate losses. Thick bars indicate amplifications.

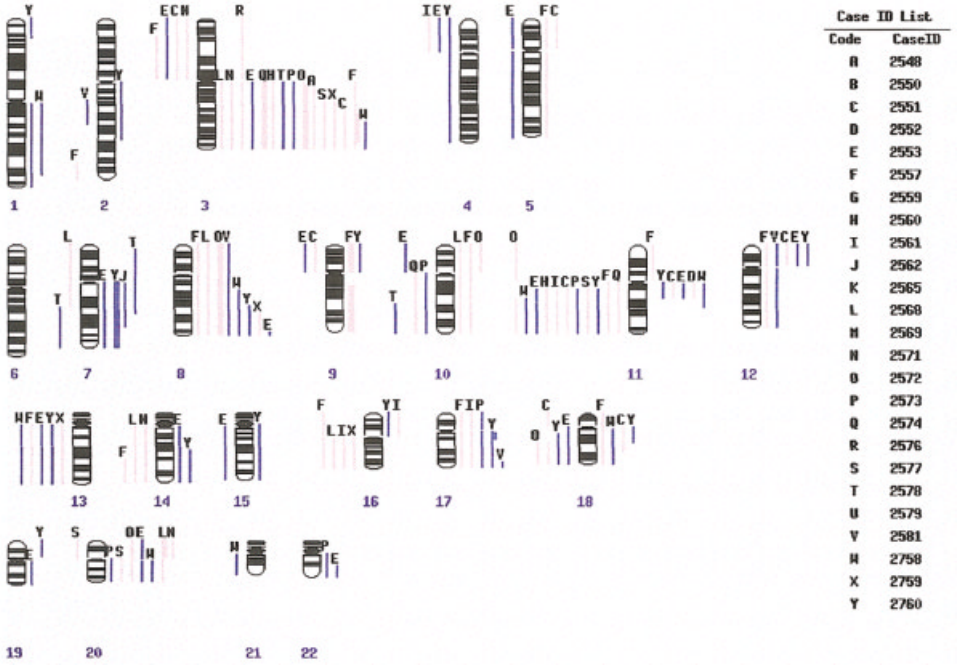
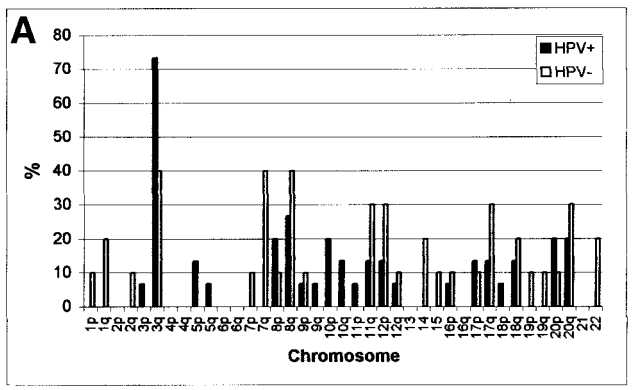


FIGURE 2 – Gains and losses in 15 HPV-positive (pink) and 10 HPV-negative (blue) squamous cell carcinomas of the tonsil. Bars to the right of the chromosomes indicate gains and those to the left, losses. Thick bars indicate amplifications.

In summary, when studying all 25 samples, gains on 3q, 8q, 20q, 11q12-q13, 12p and 17q and losses on 11q14-qter and 13q were common (Fig. 1). Furthermore, of the 15 cases with a gain on chromosome 3q, 9 (of which 3 had amplification of 3q24-q27) also had loss of chromosome 11q14-q25 (Table III). Finally, some distinct features of the karyograms of the HPV-positive and -negative groups could be identified (Table III). Significantly more HPV-positive tumors (73%) compared to -negative tumors (40%) had gain of 3q24-qter, ($p = 0.049$, Fisher's exact 2-tailed test). Only HPV-negative tumors had gain of 7q11.1-q22 ($p = 0.017$, Fisher's exact 2-tailed test), and one of the samples even showed amplification of the entire chromosome arm.

Clinical outcome

The survival analysis included only patients with at least 2 years of follow-up data and who had received treatment. For some patients, we have follow-up data for less than 2 years since they were diagnosed 1993–2001. Three patients refused treatment or were only given palliative treatment, 3 other patients received only radiotherapy and one patient did not receive any radiotherapy but had surgery. Twelve of the 15 HPV-positive patients (80%) and 3/10 HPV-negative patients (30%) survived ($p = 0.005$, Fisher's exact 2-tailed test), and the disease-specific survival for the 12 patients included in the survival analysis was significantly better for patients with HPV-positive compared to -negative tumors (Fig. 4) ($p = 0.002$, log



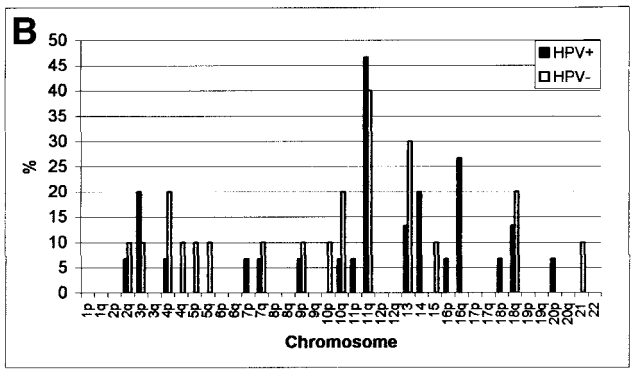


FIGURE 3 – Percentage of cases in each tumor group (15 in the HPV-positive group and 10 in the HPV-negative group) that show gains *(a)* or losses *(b)* on each chromosomal arm or band on the arm. HPV-positive cases are shown in black and HPV-negative cases in white.

TABLE III – DIFFERENCES OF GAINS AND LOSSES IN HPV-POSITIVE AND -NEGATIVE SQUAMOUS CELL CARCINOMA OF THE TONSIL

	+3q	+7q	−11q	+3q/−11q
All cases	15 (60%)	4 (16%)	11 (44%)	9 (36%)
HPV^+ cases	11 (73%)	0	7 (47%)	6 (40%)
HPV^- cases	4 (40%)	4 (40%)	4 (40%)	3 (30%)

rank). Further statistical analysis on additional variables was not meaningful due to the very limited number of available patients.

DISCUSSION

Patients with HPV-positive squamous cell carcinoma of the tonsil generally have a better overall survival compared to patients with an HPV-negative tonsillar cancer.[8] To identify additional predictive biomarkers, we screened tumors for possible genetic differences between HPV-positive and -negative tonsillar cancer using CGH.

Twenty-five primary tonsillar carcinomas were analyzed by CGH, and 15 (60%) of these tumors were HPV-positive, and 14 typed as HPV-16, which is similar to previous observations.[5,7,9,11]

In line with other studies of head-and-neck cancer, the karyograms were complex and genomic imbalances were mapped to almost all chromosomes.[25] The pattern of DNA gains and losses, however, was not random: common chromosomal imbalances in the entire material were gains of chromosomes 3q24-qter, 8q23-qter, 20q, 11q12-q13, 12p and 17q and losses of chromosomes 11q14-q25 and 13 (Fig. 1). The gains of 3q and 8q in this explicit

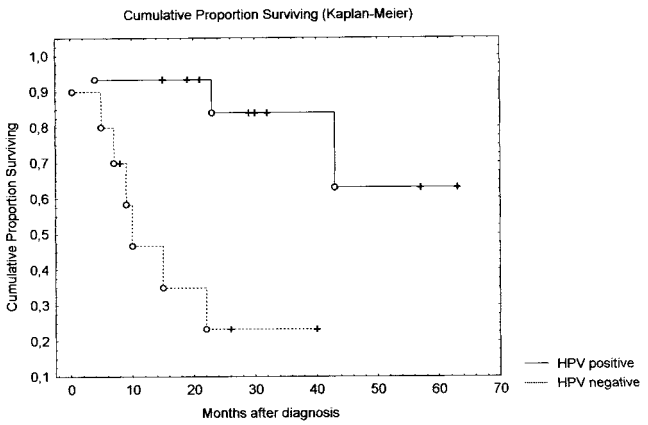


FIGURE 4 – Kaplan-Meier graph showing significantly better disease-specific survival for patients with an HPV-positive tumor compared to patients with an HPV-negative tumor ($p = 0.05$, log-rank test).

study of tonsillar cancer are similar to previous observations in head-and-neck cancers.[22] Nevertheless, loss of chromosome 3p, which is frequently found in reports on head-and-neck cancer,[32] was found in only 16% of the tumors in our study. This discrepancy was most probably due to the fact that our study exclusively comprised tonsillar tumors, whereas published reports have included tumors from different locations of the head and neck.[18] Furthermore, gain of 11q12-q13 is common in HNSCC, and several putative oncogenes map to this region.[33]

In addition to the changes that could be observed in the entire material, there were 2 statistically significant differences when comparing HPV-positive- and -negative tumors separately. These discrepancies could not be attributed to differences in tumor stage or histopathologic grade since the 2 groups were homogeneous in this respect (Table I). One profound difference was the frequency of gains on the long arm of chromosome 3, where 73% of the HPV-positive compared to 40% of the HPV-negative samples exhibited a gain in copy number DNA ($p = 0.049$, Fisher's exact 2-tailed test). The minimal affected region was 3q24-qter, which also appeared amplified in 3 HPV-positive samples, while no amplification was seen in the HPV-negative group. Notably, the frequent gain on 3q, particularly in the HPV-positive samples, argues in favor of HPV as a possible etiologic agent in tonsillar cancer since gain of 3q is a frequent as well as early event in cervical cancer,[34] where the role of HPV is undisputed.[35] At least 2 potential candidate genes have been identified on chromosome arm 3q. Firstly, Redon *et al.*[36] concluded after high-resolution amplicon mapping and transcriptional analysis that *cyclin L*, mapped to 3q25, is likely to be involved in head-and-neck cancers. Secondly, the RNA component of the human telomerase gene, *hTERC*, maps to chromosome band 3q26. The fact that this particular genomic imbalance occurs more frequently in HPV-positive tumors could point to this gene as a reasonable candidate as Veldman *et al.*[37] demonstrated a functional interaction of HPV proteins with telomerase.

A second significant difference between the HPV-positive and -negative groups was observed on chromosome 7q, where 40% of the HPV-negative samples revealed copy number increases. HPV-positive tumors never showed this specific imbalance ($p = 0.017$, Fisher's exact 2-tailed test). The minimal region affected on this chromosome was 7q11.1-q22. Differences between HPV-positive and -negative tumors are shown in Figures 2 and 3.

Only patients who received treatment and on whom at least 2 years of follow-up data were available were included in the survival analysis. Most recurrences in tonsillar cancer occur within 2 years after diagnosis, suggesting that a 2-year survival may give an indication of the clinical outcome. As expected, and similar to previous studies,[4,8,10] patients with an HPV-positive tumor had a

statistically significant better disease-specific survival ($p = 0.002$, log-rank test) than patients with an HPV-negative tumor. The most common changes, *e.g.*, gain on 3q or 8q, loss on 11q or 13 (both irrespective of HPV status or HPV status considered) and gain on chromosome 7q in the HPV-negative tumors, did not have any influence on prognosis. However, the number of cases in each subgroup was limited.

In conclusion, our data clearly show that infection with HPV has an impact on the acquisition and maintenance of specific chromosomal gains and losses.

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