EUROPEAN JOURNAL OF CANCER 43 (2007) 520–526

available at www.sciencedirect.com

journal homepage: www.ejconline.com

EGFR mutations and human papillomavirus in squamous cell carcinoma of tongue and tonsil ☆

Im Il Na[a,], Hye Jin Kang[a], Soo Youn Cho[b], Jae Soo Koh[b], Jin Kyung Lee[c], Byeong Cheol Lee[d], Guk Haeng Lee[d], Yong Sik Lee[d], Hyung Jun Yoo[e], Baek-Yeol Ryoo[a], Sung Hyun Yang[a], Yoon Sang Shim[d]*

[a]*Department of Internal Medicine, Korea Cancer Centre Hospital, Korea Institute of Radiological and Medical Sciences, 215-4, Gongneung-dong, Nowon-gu, Seoul, 139-706, Korea*
[b]*Department of Pathology, Korea Cancer Centre Hospital, Korea Institute of Radiological and Medical Sciences, 215-4, Gongneung-dong, Nowon-gu, Seoul, 139-706, Korea*
[c]*Department of Laboratory Medicine, Korea Cancer Centre Hospital, Korea Institute of Radiological and Medical Sciences, 215-4, Gongneung-dong, Nowon-gu, Seoul, 139-706, Korea*
[d]*Department of Otolaryngology-Head and Neck Surgery, Korea Cancer Centre Hospital, Korea Institute of Radiological and Medical Sciences, 215-4, Gongneung-dong, Nowon-gu, Seoul, 139-706, Korea*
[e]*Department of Radiation Oncology, Korea Cancer Centre Hospital, Korea Institute of Radiological and Medical Sciences, 215-4, Gongneung-dong, Nowon-gu, Seoul, 139-706, Korea*

ARTICLE INFO

Article history:
Received 14 August 2006
Received in revised form 1 September 2006
Accepted 7 September 2006

Keywords:
Human papillomavirus
Head and neck cancer
Tonsillar cancer
Tongue cancer
EGFR
Mutation

ABSTRACT

This study was performed to determine the clinical significance of mutations in the *EGFR* (epidermal growth factor receptor) along with their association with human papillomavirus (HPV) infections in patients with squamous cell carcinoma of the head and neck (HNSCC). Exons 18–21 of the *EGFR* tyrosine kinase domain were sequenced and HPV typing was carried out using the HPV DNA chip in tissues obtained from patients with tongue and tonsil cancer. Univariate and multivariate analyses were used to identify the significant factors. One hundred and eight patients were enrolled. Ten patients (9%) were HPV positive and 17 (16%) had *EGFR* mutations. None of the patients with *EGFR* mutations were HPV positive. Gender, age (<60 years versus ⩾60 years), and smoking history were not associated with *EGFR* mutations. A higher percentage of patients with tonsillar cancer were HPV positive than those with tongue cancer (26% and 0%, respectively; $P < 0.001$). *EGFR* mutations were not a significant prognostic factor ($P = 0.746$). HPV-positive patients had prolonged survival ($P = 0.025$). Multivariate analysis revealed a longer overall survival in HPV-positive patients ($P = 0.007$). *EGFR* mutations are not associated with the HPV-positive status, which may confer a better survival outcome. Clinical features of lung cancer patients with *EGFR* mutations were not observed in HNSCC. A further study will be needed to confirm these results.

☆ This work was presented at the 42nd annual meeting of the American Society of Clinical Oncology.

* *Corresponding author:* Tel.: +82 2 970 1228; fax: +82 2 970 2410.
E-mail address: hmona@kcch.re.kr (I.I. Na).

0959-8049/$ - see front matter
doi:10.1016/j.ejca.2006.09.025

1. Introduction

Squamous cell carcinoma of the head and neck (HNSCC) is the eighth most common cause of cancer death worldwide.[1] Smoking and alcohol consumption are well-established risk factors. However, a small portion of patients with head and neck cancer are nonsmokers and nondrinkers.[2,3] Recent studies have suggested that a human papillomavirus (HPV) infection is a risk factor for HNSCC.[4] In addition, some authors have reported that an association between a HPV infection and nonsmokers.[5] The association between a HPV infection and a carcinoma of the head and neck varies according to the anatomical site of the primary tumour. Previous studies have shown a strong association between tonsil cancer and a HPV infection.[4–7]

Some investigators have reported *EGFR* mutations in a subset of patients with non-small-cell lung cancer (NSCLC).[8,9] Among several factors, never smoking was associated with the presence of *EGFR* mutations in NSCLC patients.[8,9] However, the etiology of these *EGFR* mutations in NSCLC is unknown. Taiwanese studies have suggested that a HPV 16/18 infection might be associated with this type of cancer in nonsmoking, female lung cancer patients.[10] These findings suggest a possible association between *EGFR* mutations and a HPV infection. In addition, some patients with head and neck cancer were responsive to tyrosine kinase inhibitors (TKI) such as gefitinib.[11] Another study reported the presence of *EGFR* mutations in three out of 41 patients with head and neck cancer.[12] However, the clinical impact of *EGFR* mutations in head and neck cancer is not completely understood.

This study tested the hypothesis that a HPV infection is related to *EGFR* mutations in head and neck cancer. To accomplish this, the prevalence and association of *EGFR* mutations and HPV infections were evaluated in a subset of patients with head and neck cancer. Patients with tongue and tonsil cancer were selected because previous studies have suggested they have a different association with a HPV infection.[4,5,7] The data on *EGFR* mutations and a HPV infection was compared with the clinical features of the patients.

2. Patients and methods

2.1. Patients

Patients with squamous cell carcinoma of the tongue and tonsil were identified from the database of the Korea Cancer Centre Hospital (Seoul, Korea). The formalin-fixed paraffin-embedded tissues from 110 patients who underwent a local treatment modality such as surgery and radiation from July 1994 to December 2003 were examined. Patients who presented with a distant metastasis were excluded. Two pathologists reviewed all histology samples. Two cases with a sarcoma were excluded. The extent of the disease was assessed using clinical staging. The institutional review board of the Korea Cancer Centre Hospital approved this study.

2.2. Detection of HPV DNA

DNA was extracted from a formalin-fixed paraffin block with the highest percentage of tumours using a QIA amp DNA Mini kit (Qiagen, Hilden, Germany). DNA purification, HPV genotyping, and *EGFR* DNA sequencing were carried out in separate laboratories. The HPV detection methods were essentially those previously reported by An and colleagues.[13,14] The general primer pair, GPd5+/d6+ (located in L1), was used to detect the HPV DNA. Amplification HPV DNA was performed using the described method. β-globin was used as the internal control. The PCR products of all samples were detected by electrophoresis through 2% agarose gel. The positive samples were genotyped using an Easy HPV DNA CHIP®, which is a PCR-based DNA microarray system (Women Biotech Company, Seoul, Korea), according to the manufacturer's protocol. The HPV DNA chip contains 30 type-specific probes: 21 types from one group (HPV-16, HPV-18, HPV-26, HPV-30, HPV-31, HPV-33, HPV-35, HPV-39, HPV-45, HPV-51, HPV-52, HPV-53, HPV-56, HPV-58, HPV-59, HPV-61, HPV-66, HPV-68, HPV-70, HPV-73 and HPV-74) and 9 types from the other group (HPV-6, HPV-7, HPV-11, HPV-32, HPV-34, HPV-40, HPV-42, HPV-44, and HPV-55). The hybridisation signals on a HPV DNA chip were visualised using a ScanArray Lite scanner (Packhard Biochip Technologies, Boston, USA).

2.3. EGFR DNA sequencing

The DNA for EGFR sequencing was extracted from the paraffin embedded tissues using the same method used for HPV genotyping. The DNA (100 ng) was amplified in a 20 μL reaction solution containing 2 μL of 10 × buffer (Roche, Mannheim, Germany), 1.7–2.5 mmol/L $MgCl_2$, 250 μM deoxynucleoside triphosphate, 2.5 units of DNA (Roche), and 0.3 M of each primer pair for EGFR exons: exon 18, forward: 5′-TCCAAATGAGCTGGCAAGTG-3′, reverse:5′-TCCCAAACACTCAGTGAAACAAA-3′; exon 19, forward: 5′-ATGTGGCACCATCTCACAATTGCC-3′, reverse: 5′-CCACACAGCAAAGCAGAAACTCAC-3′; exon 20, forward: 5′–CATTCATGCGTCTTCACCTG-3′, reverse: 5′-CATATCCCCATGGCAAACTC-3′; exon 21, forward: 5′-GCTCAGAGCCT GGCATGAA-3′, reverse: 5′-CATCCTCCCCTGCATGTGT-3′. The fragments were amplified using the following conditions: 5 min initial denaturation at 94 °C, followed by 30 cycles of 1 min at 94 °C, 1 min at 57 °C and 1 min at 72 °C, with a final 10 min extension at 72°C. Purification and sequencing were performed as previously described.[15]

2.4. Statistical analysis

The Pearson χ^2 or Fisher's exact test was used for the categorical variables. The tests for the trend were performed using the Cochran–Armitage trend test. The overall survival was defined as the time from diagnosis to death from any cause. The survival curves were constructed using Kaplan–Meier method with an 80-month cutoff. The survival was compared using the log-rank test. Multivariate analysis was performed using the Cox proportional hazards model.

Stata version 8.2 was used for the statistical analyses. The Cox proportional hazards model was performed after first verifying that the proportional hazard assumptions had been met. Two-sided P values <0.05 were considered significant.

3. Results

3.1. Patient characteristics and treatment

Table 1 shows the baseline characteristics of the patients. Sixty (74%) patients were male. The median age was 52 years. Thirty-nine percent of patients had never smoked. Seventy (65%) patients were diagnosed with tongue cancer. The proportion of patients with stage I–II, III and IV cancer was 50%, 28%, and 22%, respectively. Forty-four (41%) and 22 (20%) patients were treated with radiation or surgery, respectively. Combined modality treatment including neoadjuvant chemotherapy (chemotherapy followed by radiation or surgery) was delivery in 42 (39%) patients. 5-fluorouracil (1000 mg/m^2 on days 1 to 5) and cisplatin (60 mg/m^2 on days 1) was delivered as neoadjuvant chemotherapy for up to three cycles[16]. No patients received TKI therapy.

3.2. HPV positivity

Eleven of the 108 (10%) patients showed positive findings for a HPV infection, and HPV 16 was identified in ten out of the 11 patients (90%). The HPV genotype could not be determined using sequence-based typing in one patient who showed no HPV DNA[17], and was thus considered HPV negative. Age (<60 years versus ⩾60 years) was not associated with a HPV infection (13% and 3%, respectively; $P = 0.100$) (Table 2). There was a similar prevalence of HPV positivity in the never-smokers and smokers (7% and 11%, respectively; $P = 0.545$). Gender was not associated with HPV positivity ($P = 0.758$). The proportion of HPV-positive patients was higher in those patients with tonsillar cancer than in those with tongue cancer (26% and 0%, respectively; $P < 0.001$). The histological grade was not associated with HPV positivity ($P = 0.185$). The prevalence of HPV-positive tumours was not associated with advanced stage (*P for trend* = 0.118). However, patients with HPV-positive tumours were more likely to have a higher N stage (*P for trend* = 0.037).

Table 1 – Patients' characteristics

Characteristic	N (%)
Age (years)	
Median	52
Range	13–83
Sex	
Male	80 (74)
Female	28 (26)
Smoking history	
Never-smoker	42 (39)
Smoker	66 (61)
Primary site	
Tongue	70 (65)
Tonsil	38 (35)
Stage	
I–II	54 (50)
III	30 (28)
IV	24 (22)
Initial Treatment	
Surgery only	44 (41)
Radiation only	22 (20)
Combined Treatment	42 (39)

3.3. EGFR mutations

EGFR mutations were identified in 17 (16%) patients (Tables 2 and 3). Six patients had deletions in exon 19. Eight patients harboured mutations in exon 20, and T790M was detected in two patients. C775Y, S784Y, F795S, Y801H, G810D, G810S, and Y813C were found in one patient each. Mutations in exon 21 were observed in three patients; P848L, L862P, and G863D. One patient carried two mutations, one in exon 20 (T790M) and one in exon 21 (P848L).

There was a similar mutation frequency in patients with tongue and tonsil cancer (14% and 18%, respectively; $P = 0.573$) (Table 2), and in males and females (16% and 14%, respectively; $P = 0.806$). The frequency of *EGFR* mutations was similar according to age (19% in patients <60 years and 8% in patients ⩾60 years; $P = 0.135$). There was a similar frequency of mutations in the never-smokers and smokers (14% and 17%, respectively; $P = 0.740$). There were no gender differences in the incidence of *EGFR* mutations (16% and 14% for males and females, respectively; $P = 0.806$). HPV positivity was not associated with *EGFR* mutations ($P = 0.151$). No patient with *EGFR* mutations was HPV positive.

3.4. Survival Outcome

By December 2005, 56 patients had died. Table 4 shows the results of univariate analysis. Age (<60 years versus ⩾60 years) was not a significant prognostic factor for the overall survival ($P = 0.831$). Gender and the primary site did not affect the overall survival ($P = 0.162$ and 0.279, respectively). The histological grade was not a significant prognostic factor ($P = 0.538$). An advanced stage conferred a worse survival outcome in terms of overall survival (*P for trend* = 0.007). The type of management did not affect the overall survival ($P = 0.105$). The never-smokers had a longer overall survival than the smokers ($P = 0.003$). Fig. 1A and B shows the overall survival according to the *EGFR* mutations and HPV positivity, respectively. The HPV positivity conferred a better survival (5-year survival rate: 44% versus 100%, $P = 0.025$). However, *EGFR* mutations was not a significant prognostic factor ($P = 0.746$).

Multivariate analysis was used to evaluate the survival data of the patients with *EGFR* mutations and a HPV infection after controlling for the stage and smoking history (Table 5). Patients with an advanced stage had a lower overall survival (*P for trend* = 0.004). Never-smokers had a better overall survival ($P = 0.011$). Compared with *EGFR* mutations, patients with HPV positivity had a longer overall survival ($P = 0.519$ and 0.007, respectively).

4. Discussion

There was no association found between *EGFR* mutations and a HPV infection in patients with tonsil and tongue cancer.

Table 2 – HPV positivity and EGFR mutations related to the clinical features

Characteristic	HPV			EGFR		
	Negative	Positive	*P*	Wild type	Mutation	*P*
Total number (%)	98 (91)	10 (9)		91 (84)	17 (16)	
Age (years)			0.100			0.135
<60	63 (87)	9 (13)		58 (81)	14 (19)	
⩾60	35 (97)	1 (3)		33 (92)	3 (8)	
Sex			0.758			0.806
Male	73 (91)	7 (9)		67 (84)	13 (16)	
Female	25 (89)	3 (11)		24 (86)	4 (14)	
Differentiation			0.185			0.225
Well to Moderate	77(93)	6 (7)		68 (82)	15 (18)	
Poorly	21(84)	4 (16)		23 (92)	2 (8)	
Smoking history			0.545			0.740
Never-smoker	39 (93)	3 (7)		36 (86)	6 (14)	
Smoker	59 (89)	7 (11)		55 (83)	11 (17)	
T stage			0.785			0.730
T1–2	75 (91)	7 (9)		68 (83)	14 (17)	
T3	12 (86)	2 (14)		12 (86)	2 (14)	
T4	11 (92)	1 (8)		11 (92)	1 (8)	
N Stage			0.037[a]			0.496
N0	62 (94)	4 (6)		57 (86)	9 (14)	
N1	24 (92)	2 (8)		20 (77)	6 (23)	
N2	12 (75)	4 (25)		14 (88)	2 (13)	
Stage			0.118[a]			0.405
I–II	51 (94)	3 (6)		47 (87)	7 (13)	
III	27 (90)	3 (10)		23 (77)	7 (23)	
IV	20 (83)	4 (17)		21 (88)	3 (13)	
Primary site			<0.001			0.573
Tongue	70 (100)	0 (0)		60 (86)	10 (14)	
Tonsil	28 (74)	10 (26)		31 (82)	7 (18)	

a *P* for trend.

Table 3 – Demographic data of the patients with EGFR mutations

No	Sex /Age	Smoking History	Site	Nucleotide change	Amino acid change
1	F/35	Never	Tonsil	del 2239–2248	del L747_E749, A750P
2	M/57	Smoked	Tonsil	del 2239–2248	del L747_E749, A750P
3	M/52	Smoked	Tonsil	del 2239–2247	del L747_E749
4	M/52	Smoked	Tonsil	del 2237–2251	del E746_T751 insA
5	M/51	Smoked	Tongue	del 2236–2250	del E746_A750
6	M/35	Smoked	Tongue	del 2235–2249	del E746_A750
7	M/54	Smoked	Tongue	2429 G > A	G810S
8	M/43	Smoked	Tongue	2369 C > T	T790M
9	M/23	Never	Tongue	2585 T > C	L862P
10	F/61	Never	Tongue	2351 C > A	S784Y
11	F/76	Never	Tongue	2384 T > C	F795S
12	M/31	Smoked	Tongue	2369 C > T, 2543 C > T	T790M P848L
13	M/51	Never	Tongue	2588 G > A	G863D
14	F/49	Smoked	Tongue	2429 G > A	G810D
15	M/49	Smoked	Tonsil	2438 A > G	Y813C
16	F/62	Never	Tonsil	2401 T > C	Y801H
17	M/53	Smoked	Tonsil	2324 G > A	C775Y

Although recent studies have reported HPV positivity and *EGFR* mutantions in HNSCC, no patients who had an *EGFR* mutation were positive to the HPV in the present study.[5,12] The etiology of *EGFR* mutations remains unknown. Although this study was not designed to assess the survival outcomes of patients with *EGFR* mutations, there was no apparent difference in the survival outcome of *EGFR* mutations.

In this study, the clinical features of NSCLC with *EGFR* mutations were not observed in tongue and tonsillar cancer patients with *EGFR* mutations. In the patients with tongue and tonsillar cancer, the gender and smoking history were not associated with *EGFR* mutations. Besides the differences in the patients' clinical features, there appeared to be a difference between the patients in this study and NSCLC patients

Table 4 – Results of univariate analysis

Characteristic	Overall survival		
	5YR(%)	95% CI	P
Age			0.831
<60 yr	51	38–63	
⩾60 yr	46	27–63	
Sex			0.162
Male	46	33–57	
Female	60	36–77	
Primary Site			0.279
Tongue	44	32–56	
Tonsil	61	41–76	
Differentiation			0.538
Well to Moderate	47	35–59	
Poorly	55	32–74	
Stage			0.007[a]
I–II	57	42–70	
III	52	29–69	
IV	32	15–51	
Management			0.105
Surgery	58	41–72	
Radiation	56	32–75	
Combined Treatment	38	22–54	
Smoking history			0.003
Never-smoker	67	50–79	
Smoker	38	24–51	
EGFR			0.746
Wild type	47	36–59	
Mutation	58	32–78	
HPV			0.025
Negative	44	33–55	
Positive	100	NR	

5YR, five-year survival rate; CI, confidence interval; NR, not reached.
a *P* for trend.

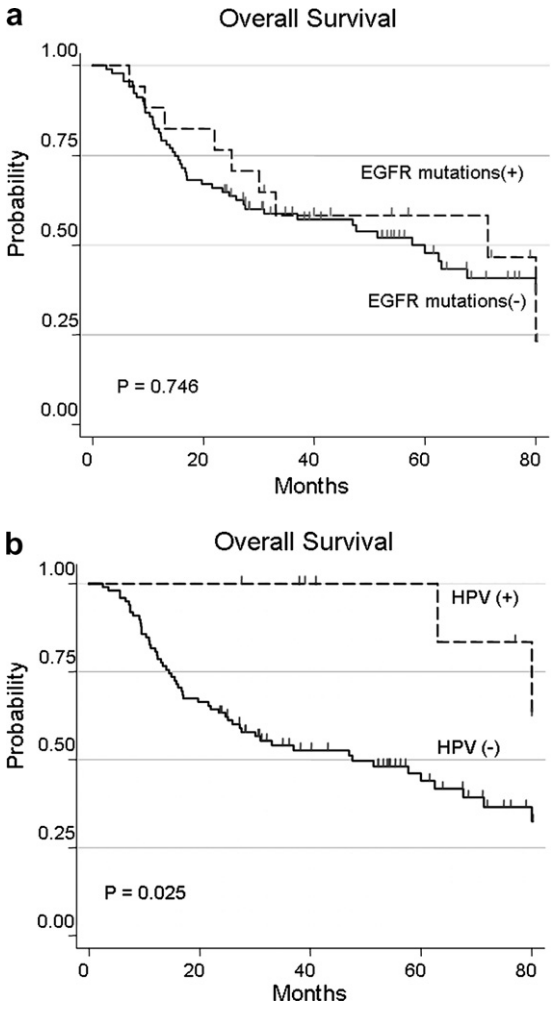

Fig. 1 – Kaplan–Meier plots of overall survival according to the presence of EGFR mutations (*A*) and HPV positivity (*B*).

in a previous report.[9] Although the deletion type of mutation in exon 19 (six patients), as reported in NSCLC, was observed, mutations in exon 20 were more common (nine patients) in the present study.[9] In our patients, all mutations in exon 20 were single nucleotide substitutions; a TKI-resistant point mutation, T790M, was found in two patients.[18] Some authors have reported a less dramatic and shorter response to TKI in patients with head and neck cancer.[11,19] Theoretically, all types of *EGFR* mutations may lead to a response to TKI in patients with NSCLC.[15,20,21] However, a recent study suggested different survival outcome according to the subtype of *EGFR* mutation.[22] The different types of *EGFR* mutation may explain the previously reported response to TKI.[11,19] Further studies will be needed to examine the prognostic impact of *EGFR* mutations in head and neck cancer, particularly in relation to TKI.

In this study, the never-smokers had a longer survival, which is consistent with previous data.[23] There was a 26% prevalence of a HPV infection in patients with tonsil cancer in this study, which is within the range reported previously.[4,5,24] The higher prevalence of HPV positivity in tonsillar cancer compared with tongue cancer is also consistent with other studies.[4,25] Several studies have reported a better survival in patients with tongue and tonsil cancer when HPV DNA is detected[26,27], as found in this study. The reason for improved survival in HPV-positive patients is unclear. Previous study reported that genotoxic treatment can reduce E6/E7 expression and induce apoptosis in HPV-positive cancer cell line.[28] Therefore, HPV-positive tumours may have an intact apoptotic response to radiation and chemotherapy.[29,30]

Some studies have reported the genotyping results using a PCR-based DNA microarray system in cervical cancer[13,14]. Genotyping using the DNA chip requires less time to run multiple PCR reactions and might be a convenient way to genotype HPV. A single genotype (HPV16) was observed in this study. However, more studies will be needed to determine its usefulness in patients with head and neck cancer.

This study showed that *EGFR* mutations were not related to the HPV positive status in HNSCC. In addition, the clinicomolecular features of *EGFR* mutations in HNSCC may be different from those in NSCLC. However, these results were collected from a relatively small sample size. Therefore, further study will be needed to confirm these findings.

Table 5 – Results of the Cox proportional hazards model

Characteristic	Overall survival		
	Hazard Ratios	95% CI	*P*
Stage			0.004[a]
I–II	1	Reference	
III	1.27	0.64–2.52	0.488
IV	2.65	1.40–4.99	0.002
Smoking History			
Never-smoker	1	Reference	
Smoker	2.27	1.21–4.27	0.011
EGFR			
Wild type	1	Reference	
Mutations	0.78	0.37–1.65	0.519
HPV			
Negative	1	Reference	
Positive	0.14	0.03–1.59	0.007

CI, confidence interval.
a *P* for trend.

Conflict of interest statement

None declared.

Acknowledgement

This work was supported in part by AstraZeneca Pharmaceuticals.

REFERENCES

1. Shibuya K, Mathers CD, Boschi-Pinto C, Lopez AD, Murray CJ. Global and regional estimates of cancer mortality and incidence by site: II. Results for the global burden of disease 2000. *BMC Cancer* 2002;**2**:37.
2. Mashberg A, Boffetta P, Winkelman R, Garfinkel L. Tobacco smoking, alcohol drinking, and cancer of the oral cavity and oropharynx among U.S. veterans. *Cancer* 1993;**72**(4):1369–75.
3. Franceschi S, Talamini R, Barra S, et al. Smoking and drinking in relation to cancers of the oral cavity, pharynx, larynx, and esophagus in northern Italy. *Cancer Res* 1990;**50**(20):6502–7.
4. Mork J, Lie AK, Glattre E, et al. Human papillomavirus infection as a risk factor for squamous-cell carcinoma of the head and neck. *N Engl J Med* 2001;**344**(15):1125–31.
5. Gillison ML, Koch WM, Capone RB, et al. Evidence for a causal association between human papillomavirus and a subset of head and neck cancers. *J Natl Cancer Inst* 2000;**92**(9):709–20.
6. Syrjanen S. HPV infections and tonsillar carcinoma. *J Clin Pathol* 2004;**57**(5):449–55.
7. Syrjanen S. Human papillomavirus (HPV) in head and neck cancer. *J Clin Virol* 2005;**32**(Suppl 1):S59–66.
8. Marchetti A, Martella C, Felicioni L, et al. EGFR mutations in non-small-cell lung cancer: analysis of a large series of cases and development of a rapid and sensitive method for diagnostic screening with potential implications on pharmacologic treatment. *J Clin Oncol* 2005;**23**(4):857–65.
9. Shigematsu H, Lin L, Takahashi T, et al. Clinical and biological features associated with epidermal growth factor receptor gene mutations in lung cancers. *J Natl Cancer Inst* 2005;**97**(5):339–46.
10. Cheng YW, Chiou HL, Sheu GT, et al. The association of human papillomavirus 16/18 infection with lung cancer among nonsmoking Taiwanese women. *Cancer Res* 2001;**61**(7):2799–803.
11. Wirth LJ, Haddad RI, Lindeman NI, et al. Phase I study of gefitinib plus celecoxib in recurrent or metastatic squamous cell carcinoma of the head and neck. *J Clin Oncol* 2005;**23**(28):6976–81.
12. Lee JW, Soung YH, Kim SY, et al. Somatic mutations of EGFR gene in squamous cell carcinoma of the head and neck. *Clin Cancer Res* 2005;**11**(8):2879–82.
13. An HJ, Cho NH, Lee SY, et al. Correlation of cervical carcinoma and precancerous lesions with human papillomavirus (HPV) genotypes detected with the HPV DNA chip microarray method. *Cancer* 2003;**97**(7):1672–80.
14. An HJ, Kim KR, Kim IS, et al. Prevalence of human papillomavirus DNA in various histological subtypes of cervical adenocarcinoma: a population-based study. *Mod Pathol* 2005;**18**(4):528–34.
15. Han SW, Kim TY, Hwang PG, et al. Predictive and prognostic impact of epidermal growth factor receptor mutation in non-small-cell lung cancer patients treated with gefitinib. *J Clin Oncol* 2005;**23**(11):2493–501.
16. Chang A, Wu H, Park C, et al. Retrospective analysis of the treatment results for patients with squamous cell carcinoma of tonsil. *Cancer Res Treat* 2005;**37**(2):92–7.
17. Wu Y, Chen Y, Li L, et al. Associations of high-risk HPV types and viral load with cervical cancer in China. *J Clin Virol* 2006;**35**(3):264–9.
18. Kobayashi S, Boggon TJ, Dayaram T, et al. EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. *N Engl J Med* 2005;**352**(8):786–92.
19. Cohen EE, Rosen F, Stadler WM, et al. Phase II trial of ZD1839 in recurrent or metastatic squamous cell carcinoma of the head and neck. *J Clin Oncol* 2003;**21**(10):1980–7.
20. Shepherd FA, Rodrigues Pereira J, Ciuleanu T, et al. Erlotinib in previously treated non-small-cell lung cancer. *N Engl J Med* 2005;**353**(2):123–32.
21. Eberhard DA, Johnson BE, Amler LC, et al. Mutations in the epidermal growth factor receptor and in KRAS are predictive and prognostic indicators in patients with non-small-cell lung cancer treated with chemotherapy alone and in combination with erlotinib. *J Clin Oncol* 2005;**23**(25):5900–9.
22. Jackman DM, Yeap BY, Sequist LV, et al. Exon 19 deletion mutations of epidermal growth factor receptor are associated with prolonged survival in non-small cell lung cancer patients treated with gefitinib or erlotinib. *Clin Cancer Res* 2006;**12**(13):3908–14.
23. Pytynia KB, Grant JR, Etzel CJ, et al. Matched-pair analysis of survival of never smokers and ever smokers with squamous cell carcinoma of the head and neck. *J Clin Oncol* 2004;**22**(19):3981–8.
24. Niedobitek G, Pitteroff S, Herbst H, et al. Detection of human papillomavirus type 16 DNA in carcinomas of the palatine tonsil. *J Clin Pathol* 1990;**43**(11):918–21.
25. Syrjanen S. Human papillomavirus (HPV) in head and neck cancer. *J Clin Virol* 2005;**32**(Suppl 1):S59–66.
26. Dahlgren L, Dahlstrand HM, Lindquist D, et al. Human papillomavirus is more common in base of tongue than in mobile tongue cancer and is a favorable prognostic factor in base of tongue cancer patients. *Int J Cancer* 2004;**112**(6):1015–9.
27. Ritchie JM, Smith EM, Summersgill KF, et al. Human papillomavirus infection as a prognostic factor in carcinomas of the oral cavity and oropharynx. *Int J Cancer* 2003;**104**(3):336–44.

28. Butz K, Geisen C, Ullmann A, Spitkovsky D, Hoppe-Seyler F. Cellular responses of HPV-positive cancer cells to genotoxic anti-cancer agents: repression of E6/E7-oncogene expression and induction of apoptosis. *Int J Cancer* 1996;**68**(4):506–13.
29. DeWeese TL, Walsh JC, Dillehay LE, et al. Human papillomavirus E6 and E7 oncoproteins alter cell cycle progression but not radiosensitivity of carcinoma cells treated with low-dose-rate radiation. *Int J Radiat Oncol Biol Phys* 1997;**37**(1):145–54.
30. Ferris RL, Martinez I, Sirianni N, et al. Human papillomavirus-16 associated squamous cell carcinoma of the head and neck (SCCHN): a natural disease model provides insights into viral carcinogenesis. *Eur J Cancer* 2005;**41**(5):807–15.